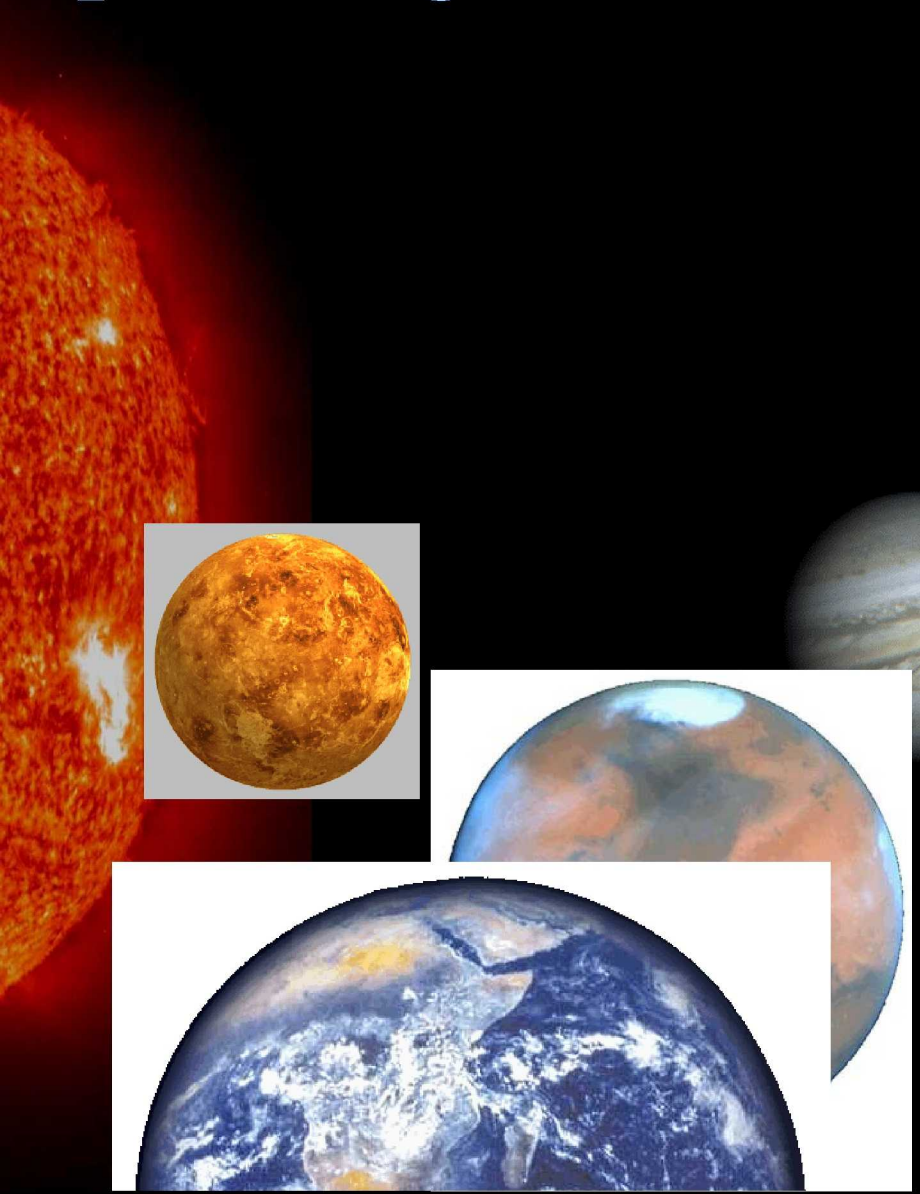
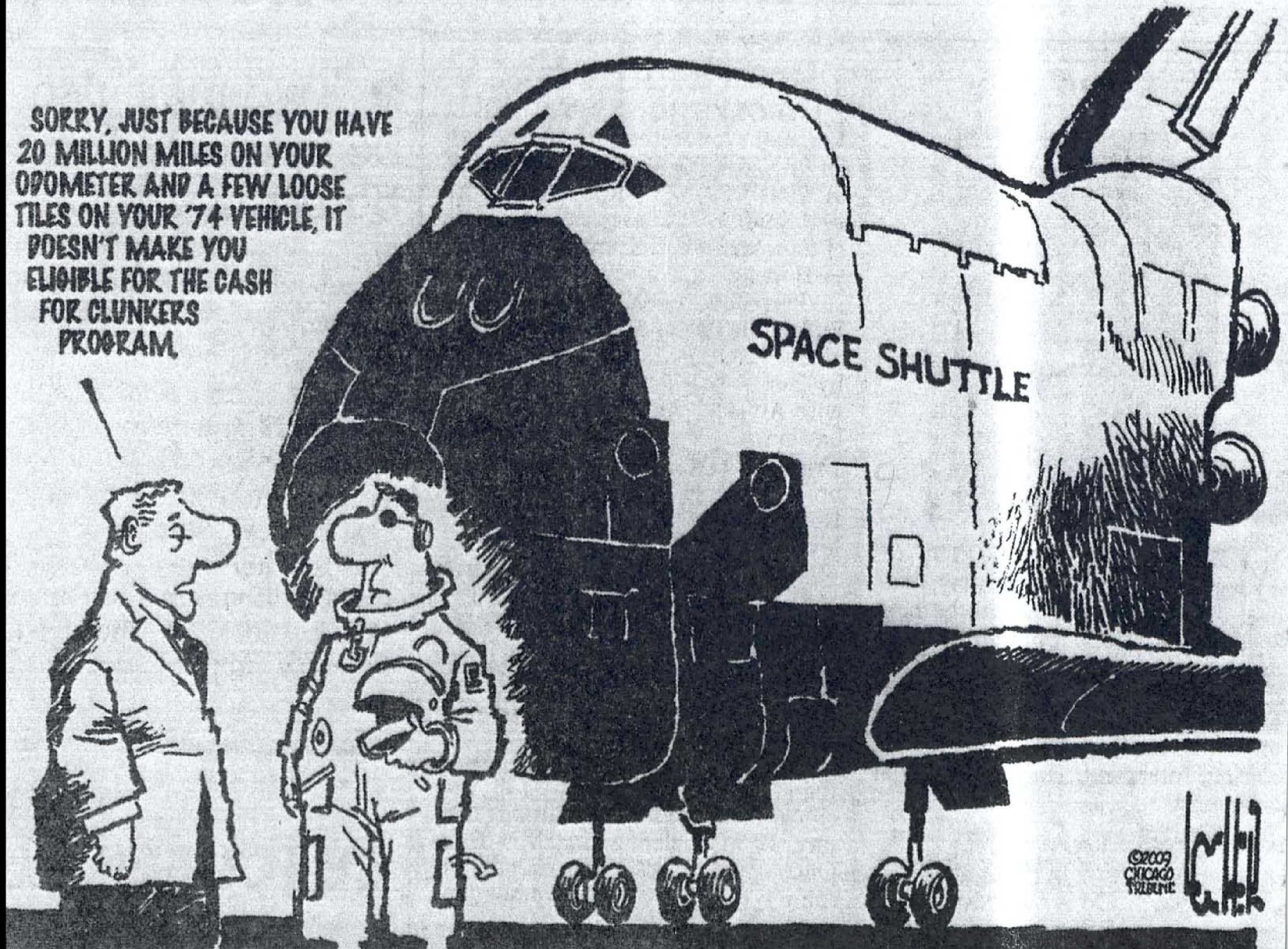
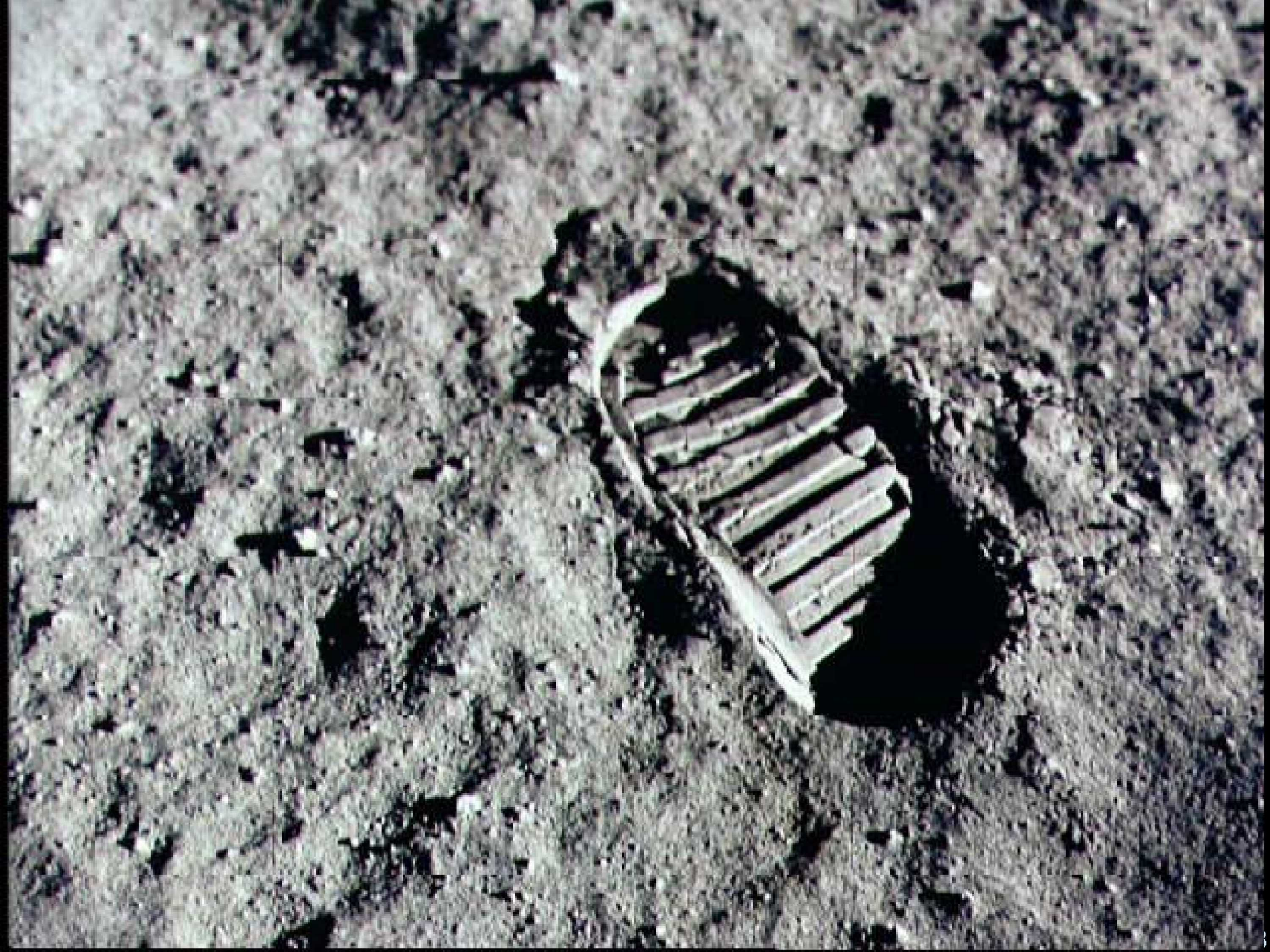
Infectious Disease Risk Associated With Space Flight



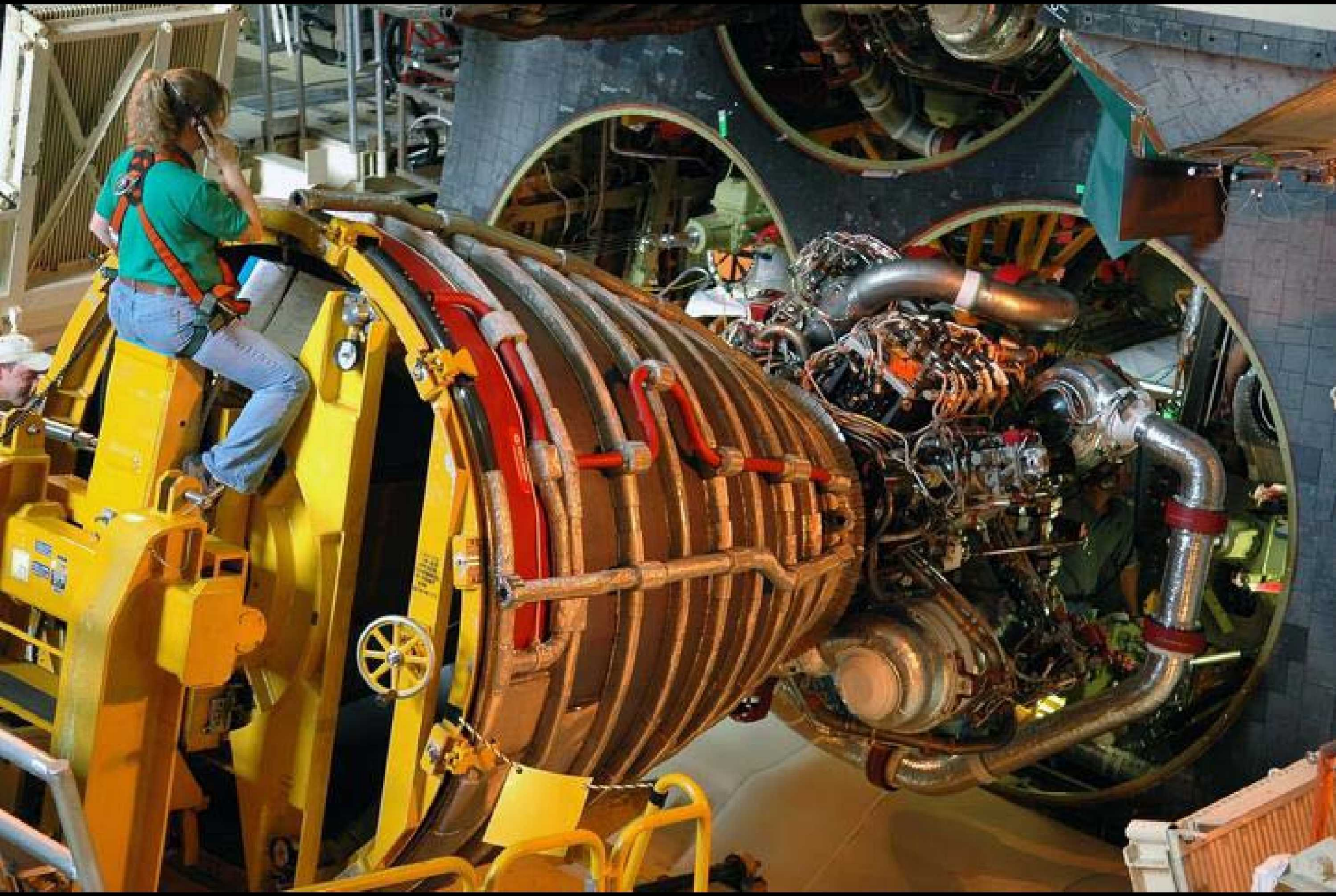
Human Exploration

Duane L. Pierson Ph.D.
NASA Johnson Space Center
April 2010


SORRY, JUST BECAUSE YOU HAVE 20 MILLION MILES ON YOUR ODOMETER AND A FEW LOOSE TILES ON YOUR '74 VEHICLE, IT DOESN'T MAKE YOU ELIGIBLE FOR THE CASH FOR CLUNKERS PROGRAM.
SPACE SHUTTLE
©2009 CHICAGO TRIBUNE



Shuttle In Sling Ready For Lift In VAB

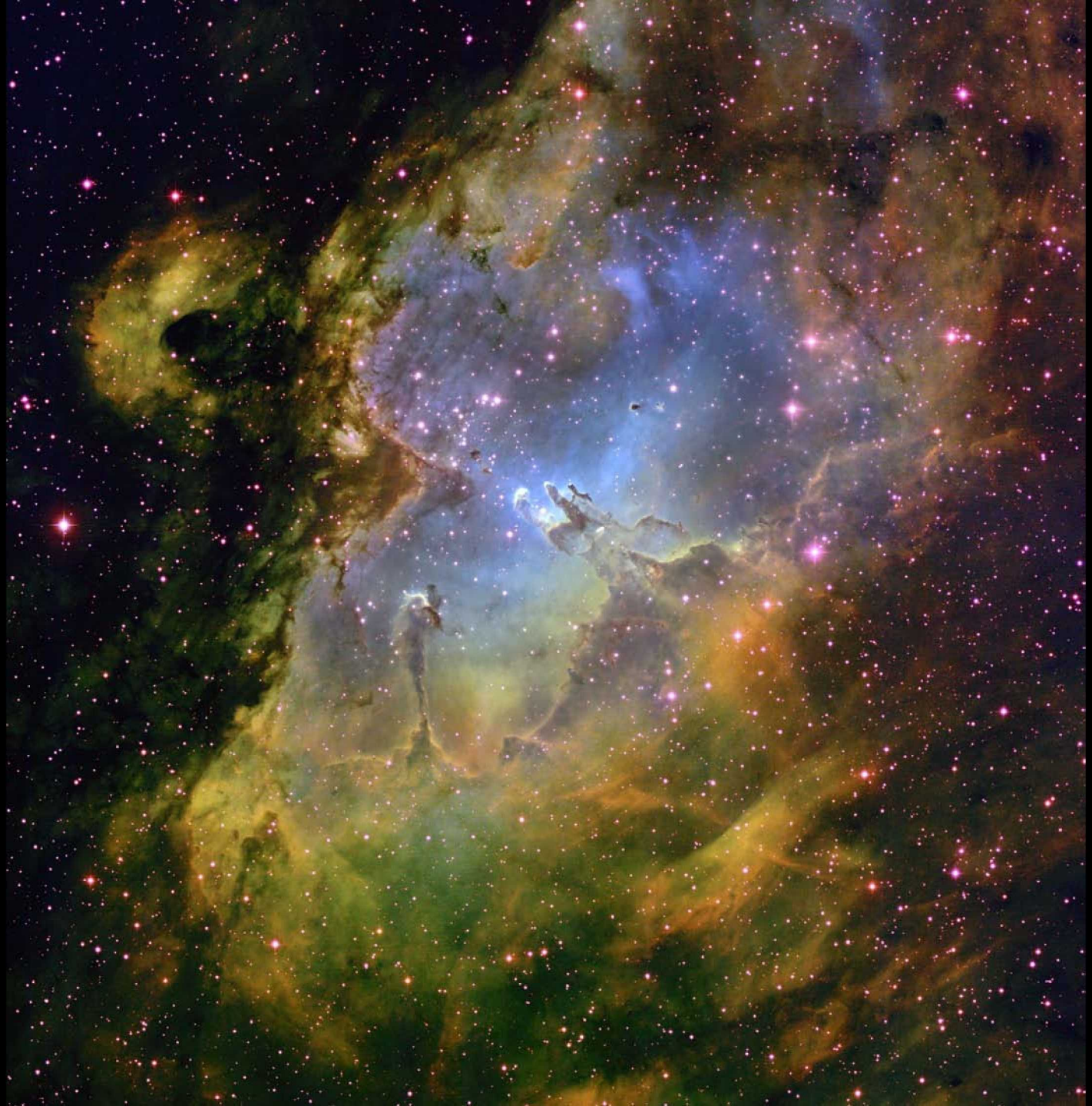


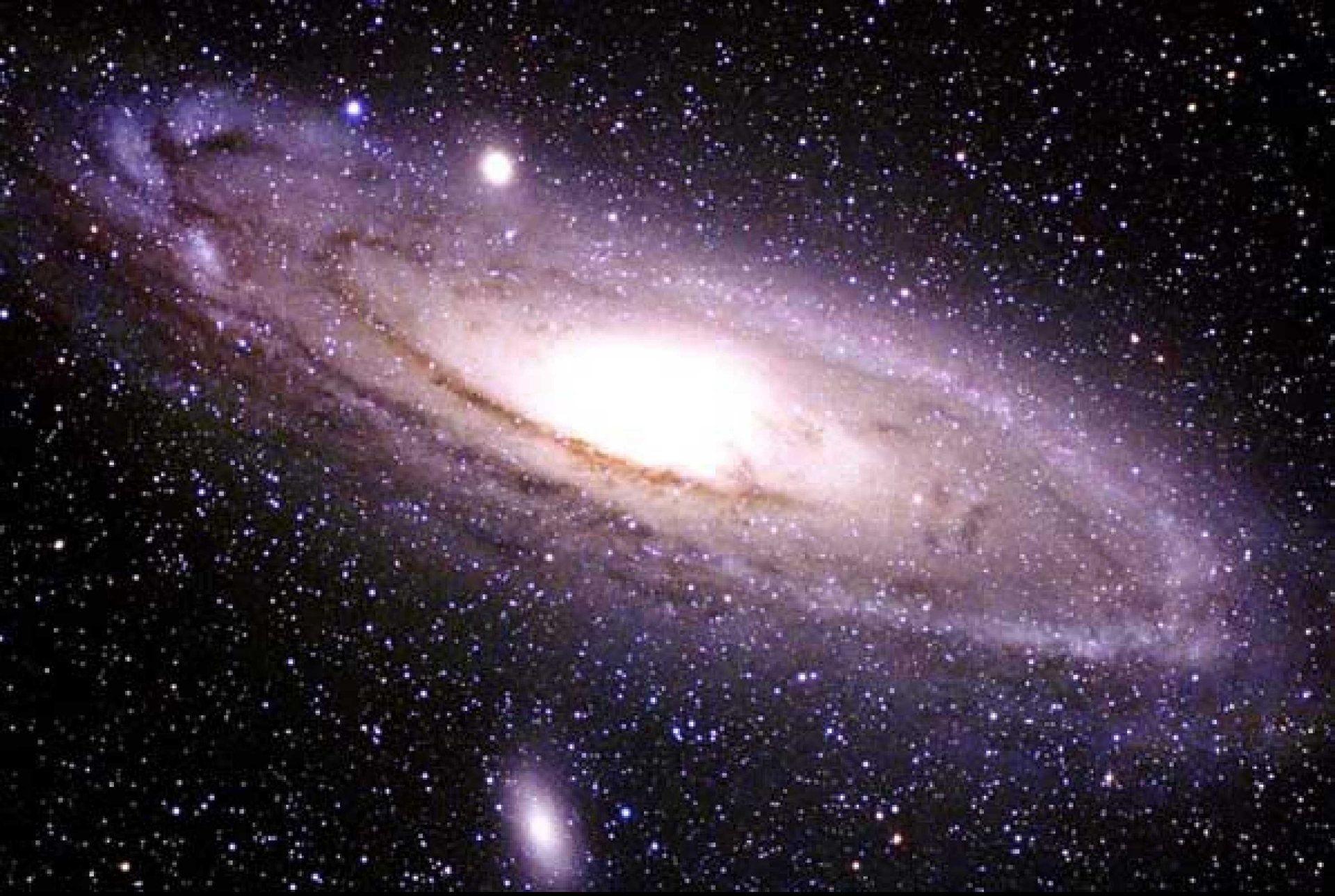
Shuttle Has Been
Moved To VAB And
And Will Be Attached
To External Tank

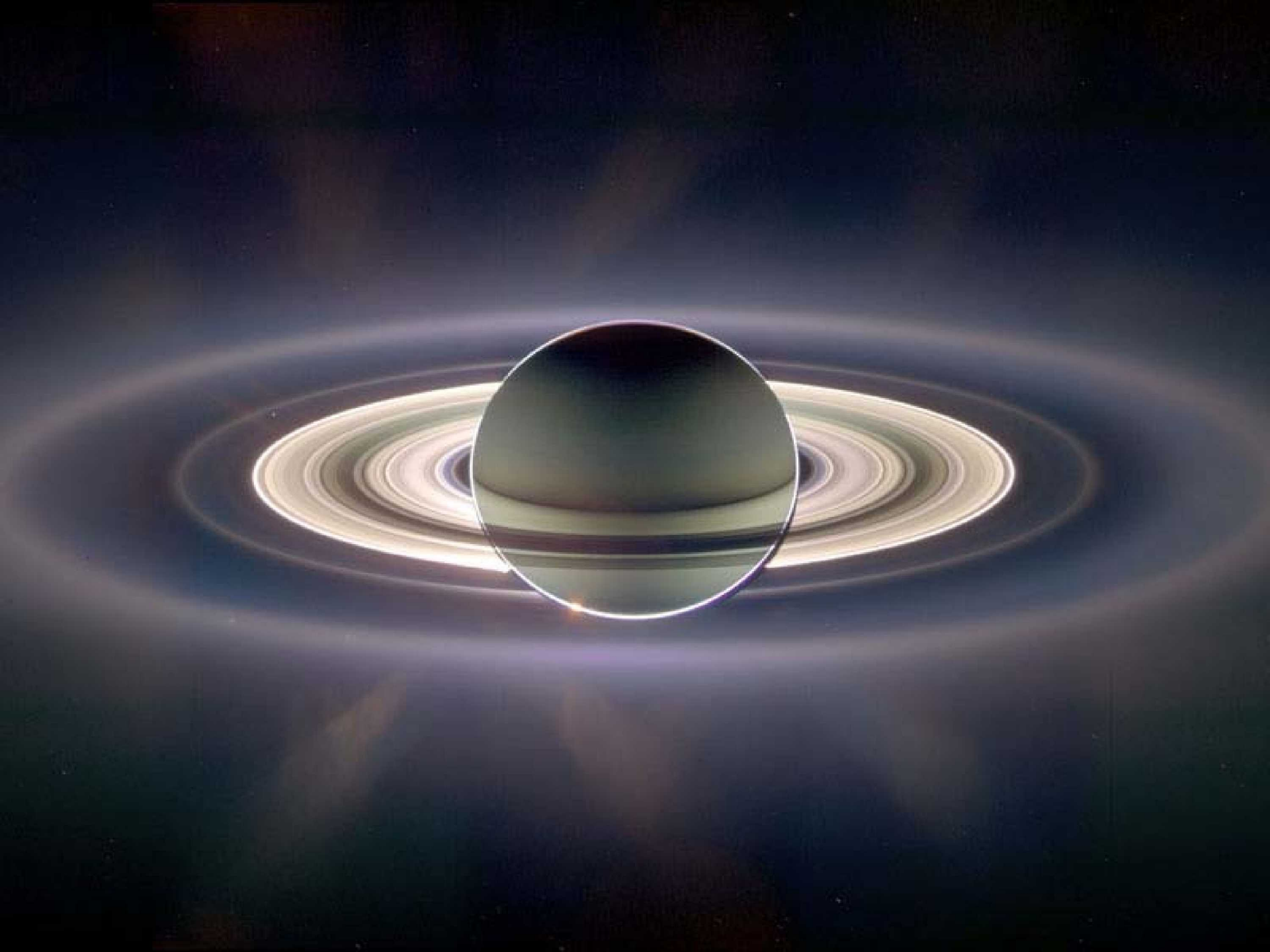

Discovery

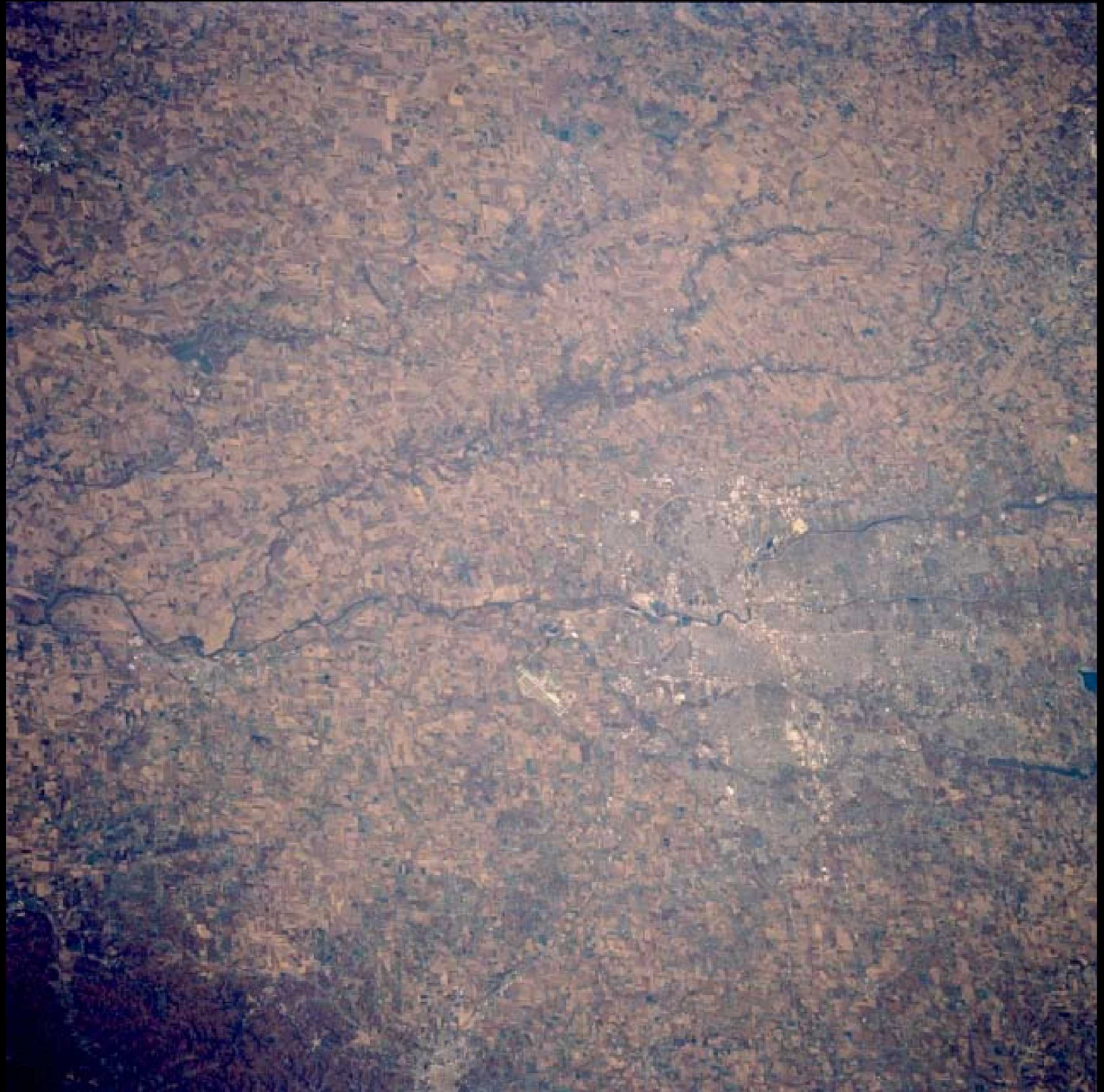

LINNEHAN
SEARFOSS
ALTMAN
STS-90
NEUROLAB
BLUFORD
PAWELCZYK
WILLIAMS


940207 185334 STS60 87 030







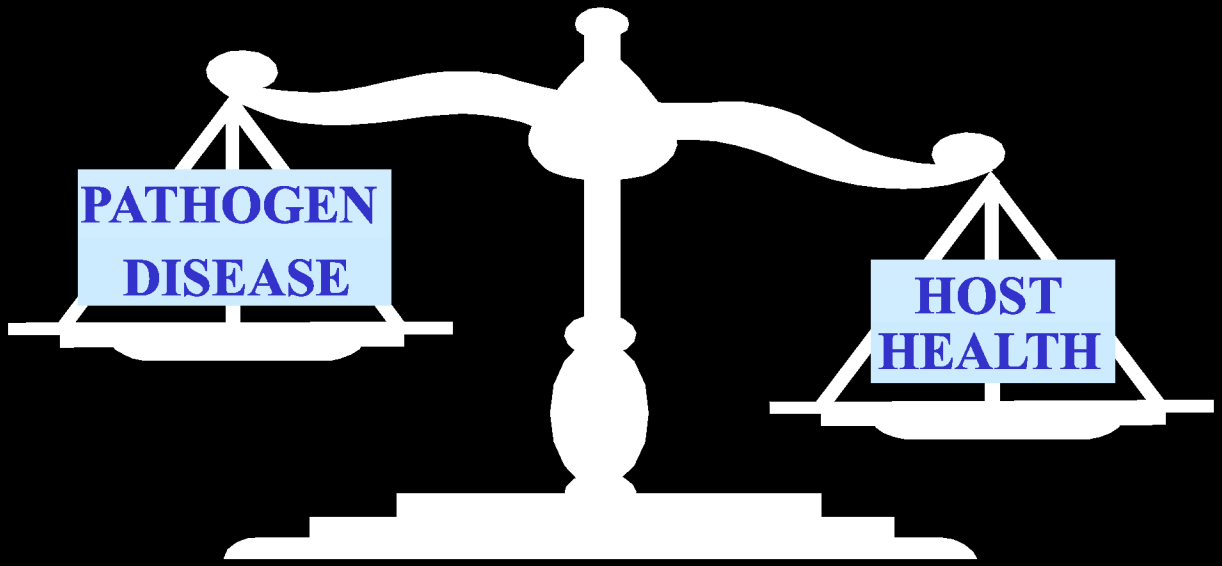


MICROBIOLOGICAL RISKS

Sources	Controls
• Crewmembers	Preflight screening, quarantine, vaccination, antimicrobials, antivirals
• Water	Preflight/inflight monitoring, biocides
• Food	Preflight analyses
• Air	Preflight/inflight monitoring, filtration
• Surfaces	Preflight/inflight monitoring, disinfection
• Payloads	Preflight cleaning, biosafety assessment, disinfection




FACTORS INCREASING DISEASE RISK



- Crowded living conditions
- Closed-loop environment (water/air)
- Reduced capability for personal hygiene
- Limited clean-up and disinfection capability
- Inability to isolate contagious crewmember
- Limited treatment capability and crew return
- Altered immune response

SPACEFLIGHT FACTORS AFFECTING INFECTIOUS DISEASE RISK

Positive Factors

- Healthy well-conditioned crew
- Preflight exams & restricted access
- No exposure to many public health pathogens
- Diagnostic/treatment on board
- Earth to orbit medical consult

Negative Factors

- Isolated/enclosed environment
- Recycled air/water (urine, humidity condensate)
- Limited diagnostics/treatment on board
- Remote location/limited return pathogens (e.g. TB, HIV, Hep A/B/C)
- Uniquely stressful environment
- Diminshed Immunity
- Increased virulence in bacteria

ADAPTATION TO SPACEFLIGHT

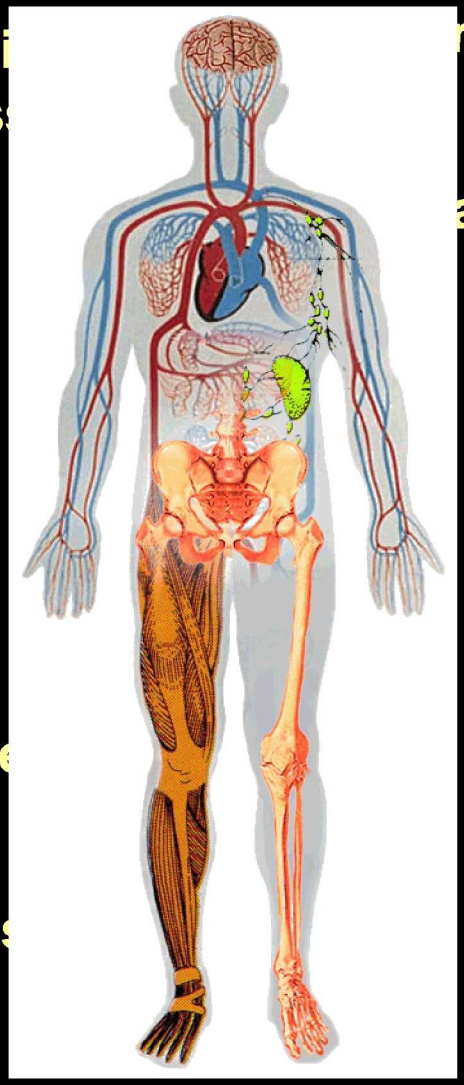
Psychological/Behavi
Performance is

Taste and odor sensitivity

Gastrointestinal alterations

Fluid shifts, hematological change

Muscle loss



rosensory adaptations

ardiovascular adaptations

Sleep and circadian rhythm disturbances

Bone loss

Immune changes

ENVIRONMENTAL FACTORS AFFECTING IMMUNITY

Anxiety

Confinement

Isolation

Physical

Psychosocial

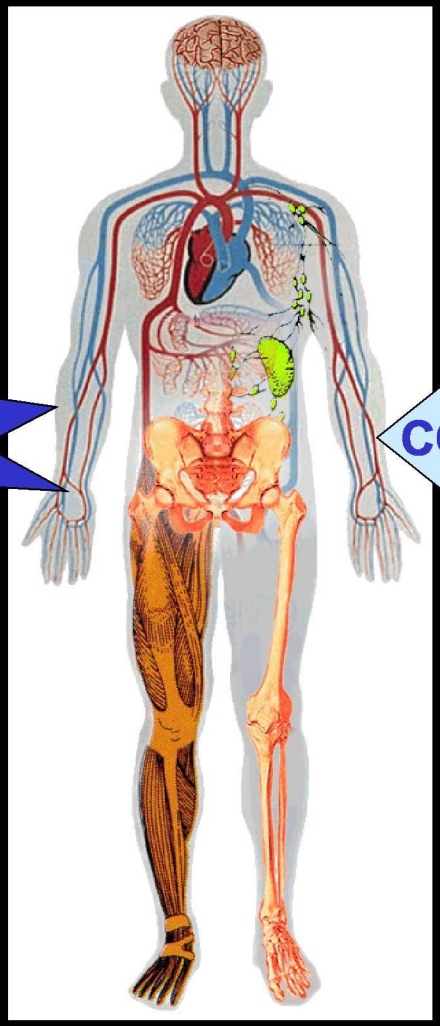
Sleep deprivation

Microgravity

Acceleration

Radiation

STRESS



CONTAMINANTS

Microorganisms

Mold spores

Chemicals

Dust mites

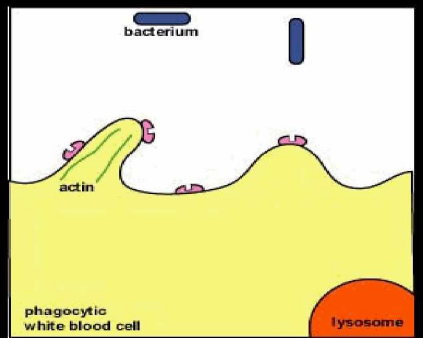
Allergens

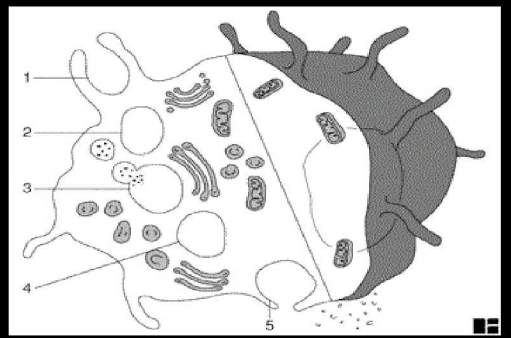
Insects

IMMUNITY

HUMAN SPACE FLIGHT IMMUNOLOGY

•White blood cell count	Increased (neutrophils)
•Lymphocyte proliferative responses	Decreased
•Cell mediated immunity	Decreased
•Cytokine production	Increased/Decreased
•Humoral factors	No Change
•Specific antibody response	No Change
•Neutrophil/Monocyte functions	Decreased
•NK cell cytotoxicity	Decreased
•Latent virus reactivation	Increased





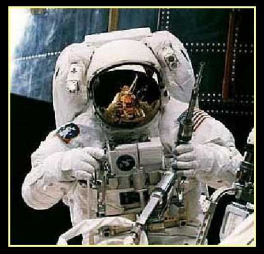
INFECTIOUS DISEASES IN ASTRONAUTS

STS-1 Through STS-108

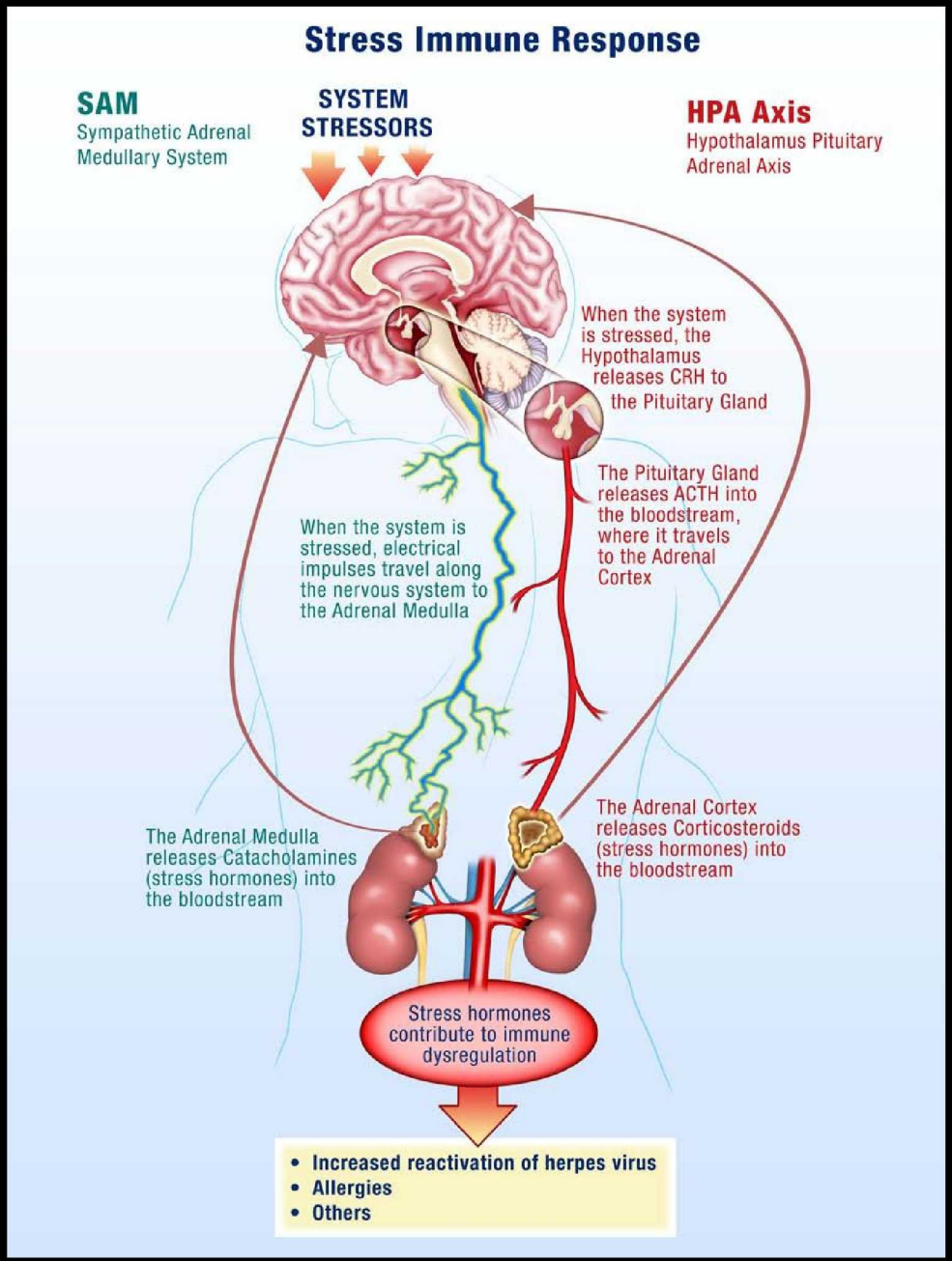
- Fungal infections
- Flu-like syndrome
- Urinary tract infections
- Aphthous stomatitis
- Viral gastrointestinal disease
- Subcutaneous skin infections
- Viral reactivation
- URI (common cold, sore throat)
- Sty

IMMUNE SYMPTOMS

- Allergic rhinitis
- Hypersensitivity
- Coughing/Sneezing
- Rashes/Skin disorders
- Infectious of cuts
- Delayed wound healing

Source: Medical Informatics & Health Care Systems Branch Epidemiology Section; July 2002

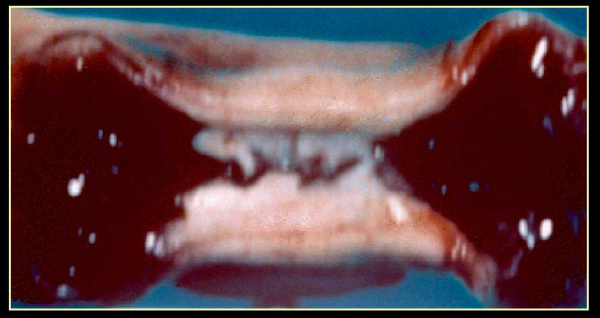

Stress Immune Response
SAM
Sympathetic Adrenal Medullary System
SYSTEM STRESSORS
HPA Axis
Hypothalamus Pituitary Adrenal Axis
When the system is stressed, the Hypothalamus releases CRH to the Pituitary Gland
The Pituitary Gland releases ACTH into the bloodstream, where it travels to the Adrenal Cortex
When the system is stressed, electrical impulses travel along the nervous system to the Adrenal Medulla
The Adrenal Medulla releases Catacholamines (stress hormones) into the bloodstream
The Adrenal Cortex releases Corticosteroids (stress hormones) into the bloodstream
Stress hormones contribute to immune dysregulation
• Increased reactivation of herpes virus
• Allergies
• Others

Why Herpes viruses?

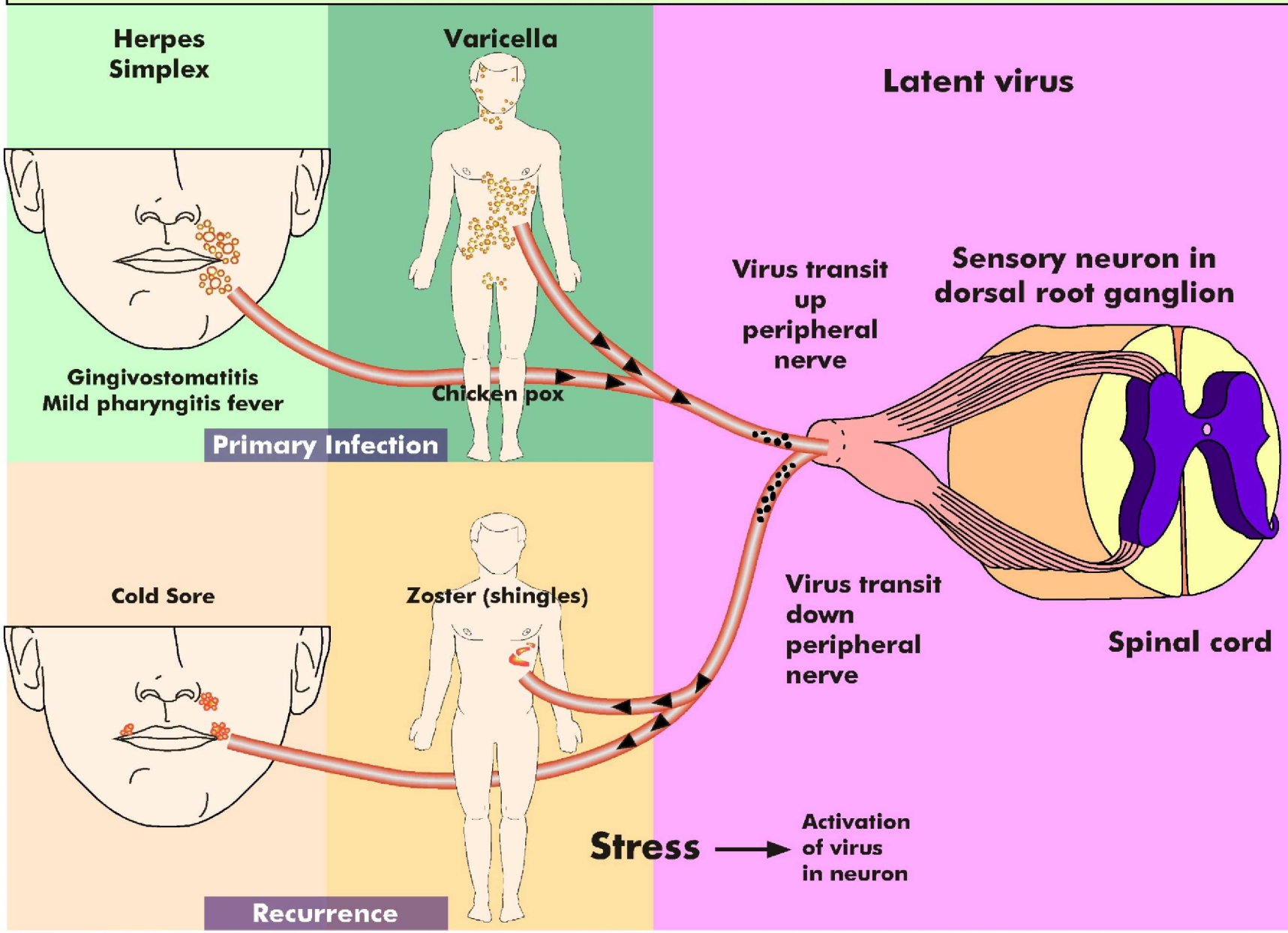
Herpesviruses are:

1. **The most readily recognized latent viruses.**
2. **Ubiquitous and represent important infectious disease risks with monogenic potential.**
3. **Not mitigated by preflight quarantine. Space flight stress alters immune response.**
4. **Diminished immunity results in reactivation & shedding of latent viruses**

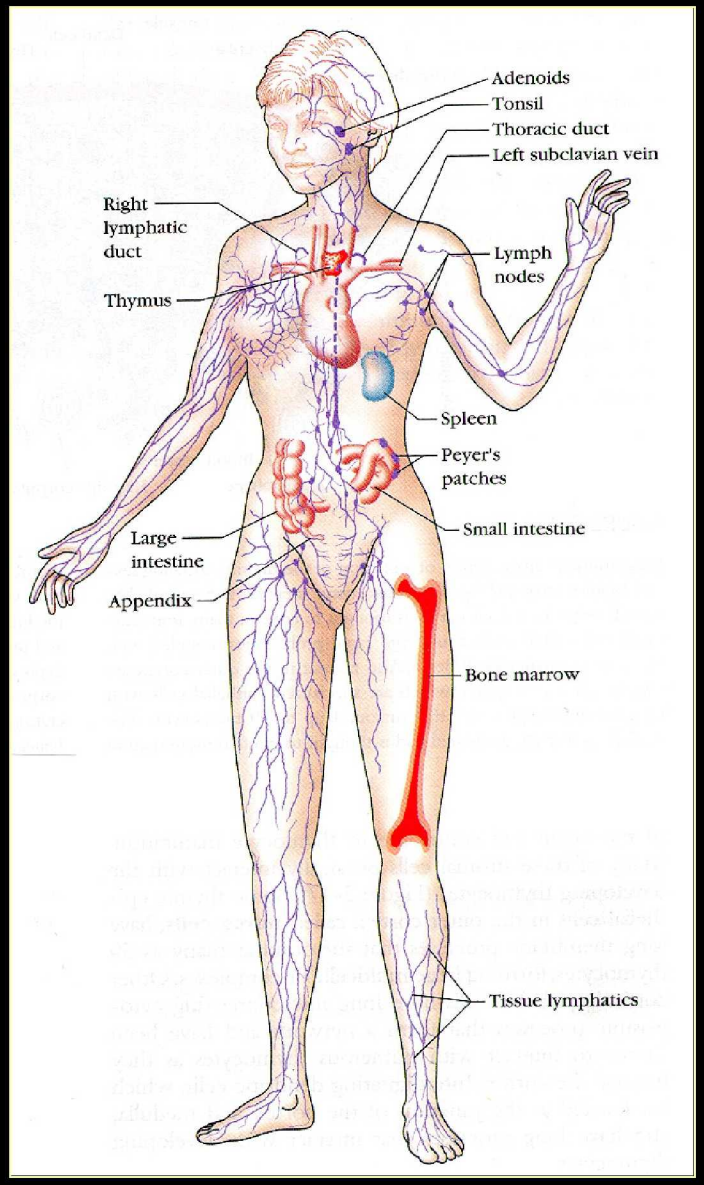
Specific Application:
May be used as an early predictor of impending medically significant changes in the immune response.



Latent Viral Reactivation

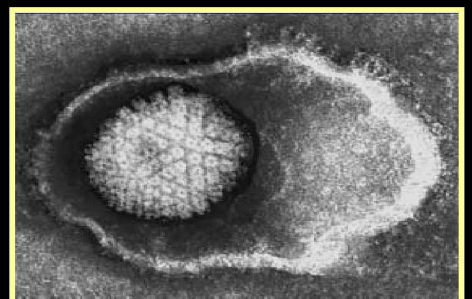


Herpes virus Infections



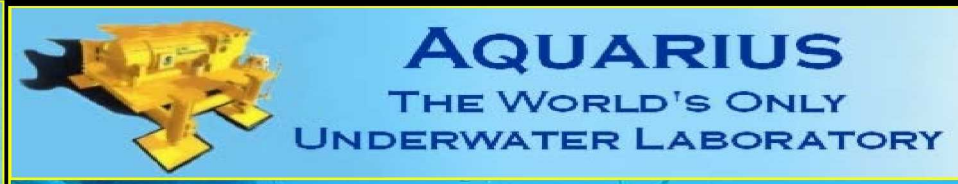
4 of 8 herpes viruses reactivate in response to spaceflight

- **Herpes Simplex Virus (HSV-**
  - **Ocular herpes, encephalitis**
- **Varicella-zoster virus (VZV)**
  - **Chicken pox, shingles**
- **Epstein-barr virus (EBV)**
  - **Mononucleosis, tumors**
- **Cytomegalovirus (CMV)**
  - **Mononucleosis, hepatitis**

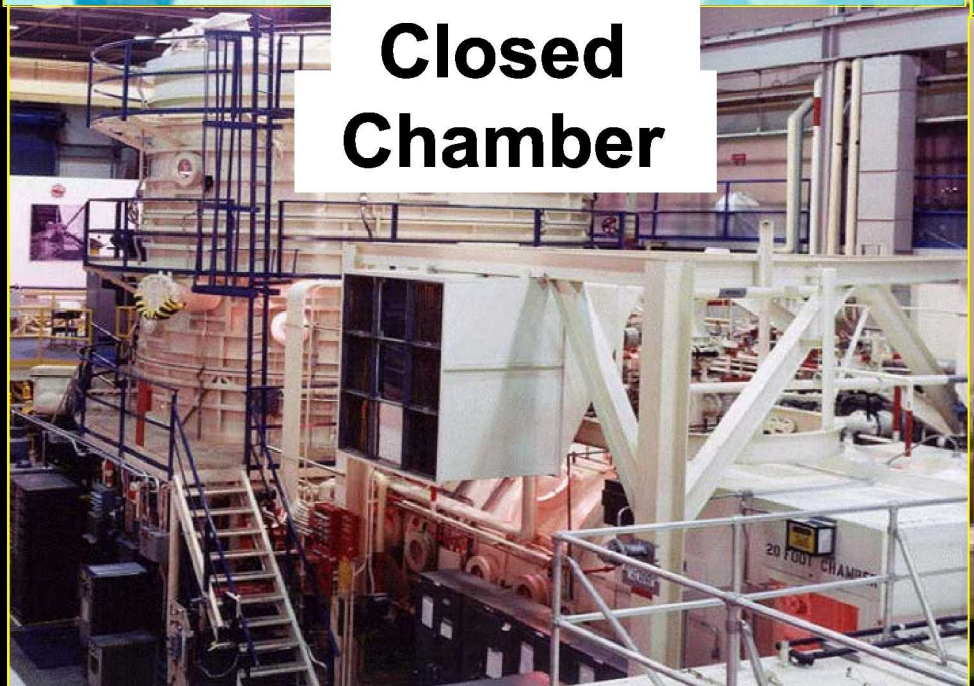


Space Analogs

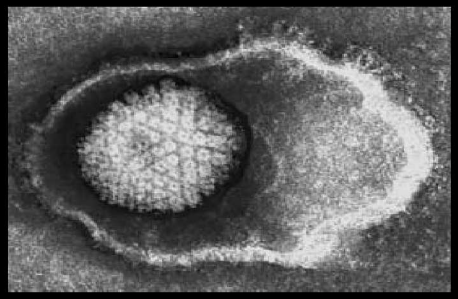
Antarctica



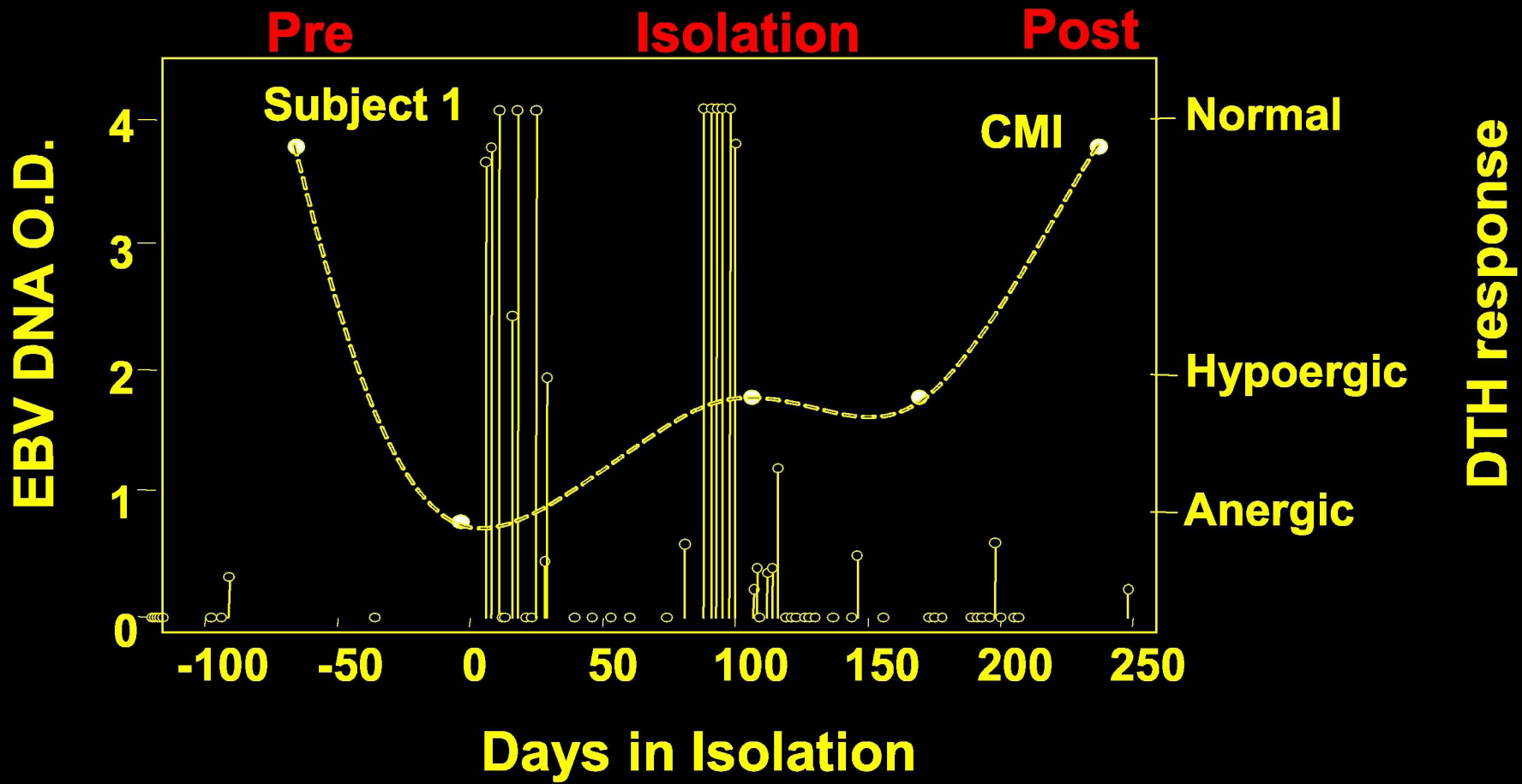
Closed Chamber



Bed Rest

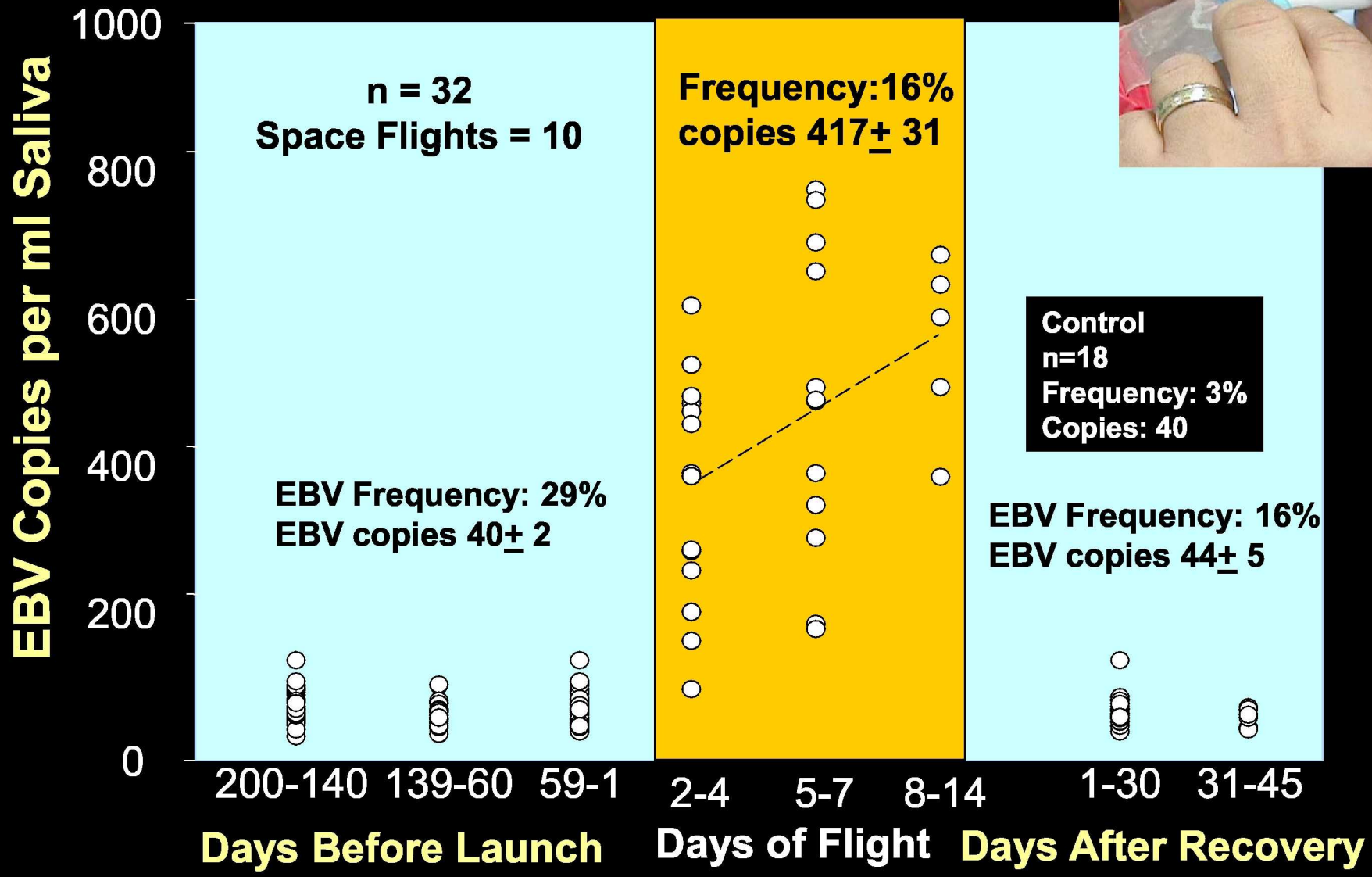


Antarctica: EBV



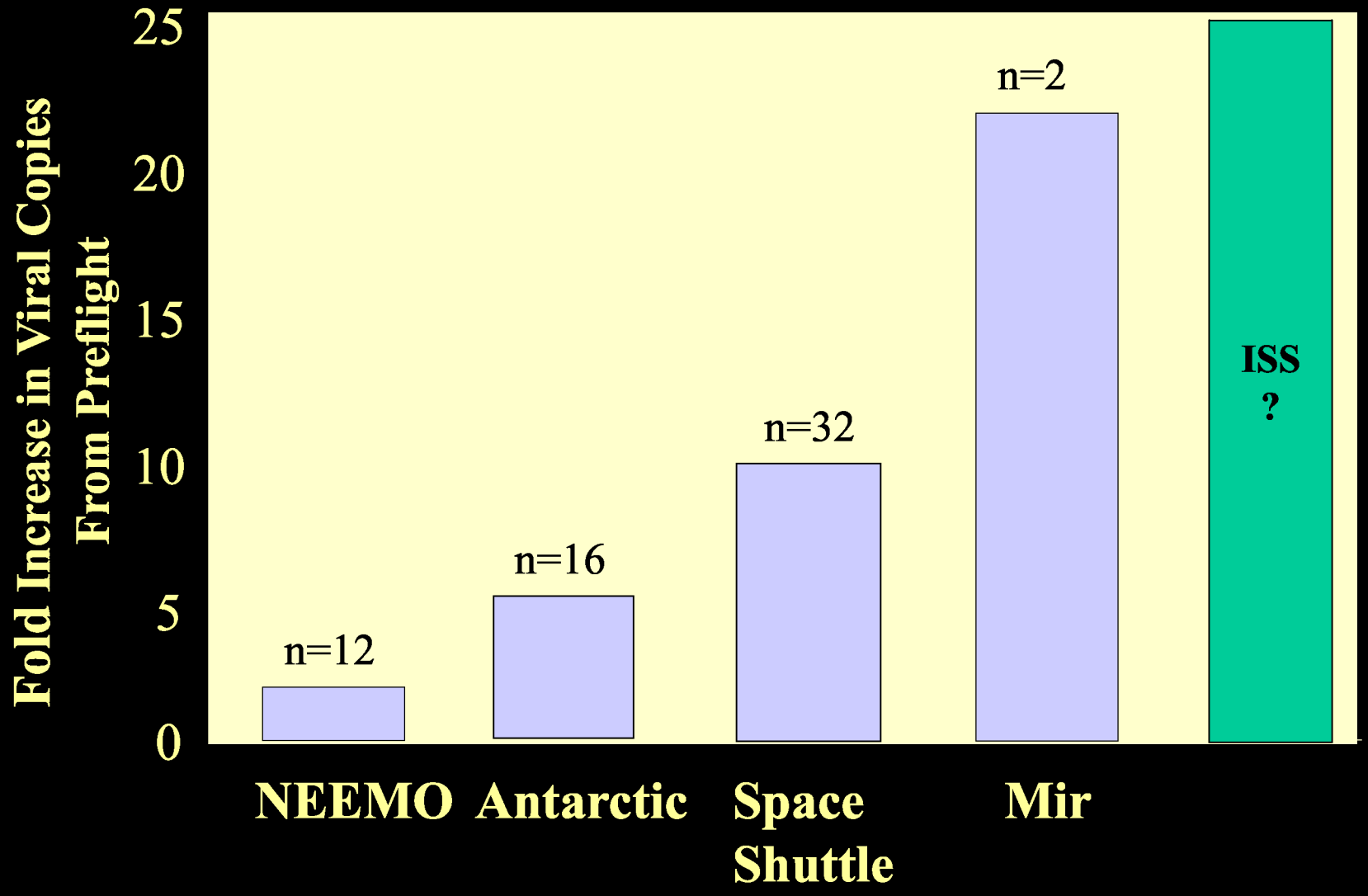
Mehta et al., J. Medical Virology 2000

Space Shuttle EBV Copies



Pierson et al., Brain Behavior & Immunity, 2005

Fold Increase In EBV Copy Numbers



Summary of Nested RT-PCR Analysis of EBV Gene Expression in Healthy Young Adults

			Latency I-III					IE/E[b] Replicative				Late Replicative	
Subject	Actin	EBER1	Qp	LMP2A	Cp/Wp	LMP1	EBNA2	BZLF1	BHRF1	SM	Fp	BALF5	gp220
1	+++												
2	+++	+++											
3	+	+											
4	+++	+											
5	+++												
6	+++	+++											
7	+++	+											
8	+	+											
9	+	+++											
10	+++												
11	+++	+						+					
12	+	+	+										
13	+++	+++	+										
14	+++	+				+							
15	+++	+											
16	+++	+											
17	+++	+++											
18	+++												
19	+++												
20	+++	+++											
21	+++	+	+										
22	+++	+++											
23	+++	+++									+		
24	+++	+++	+								+		

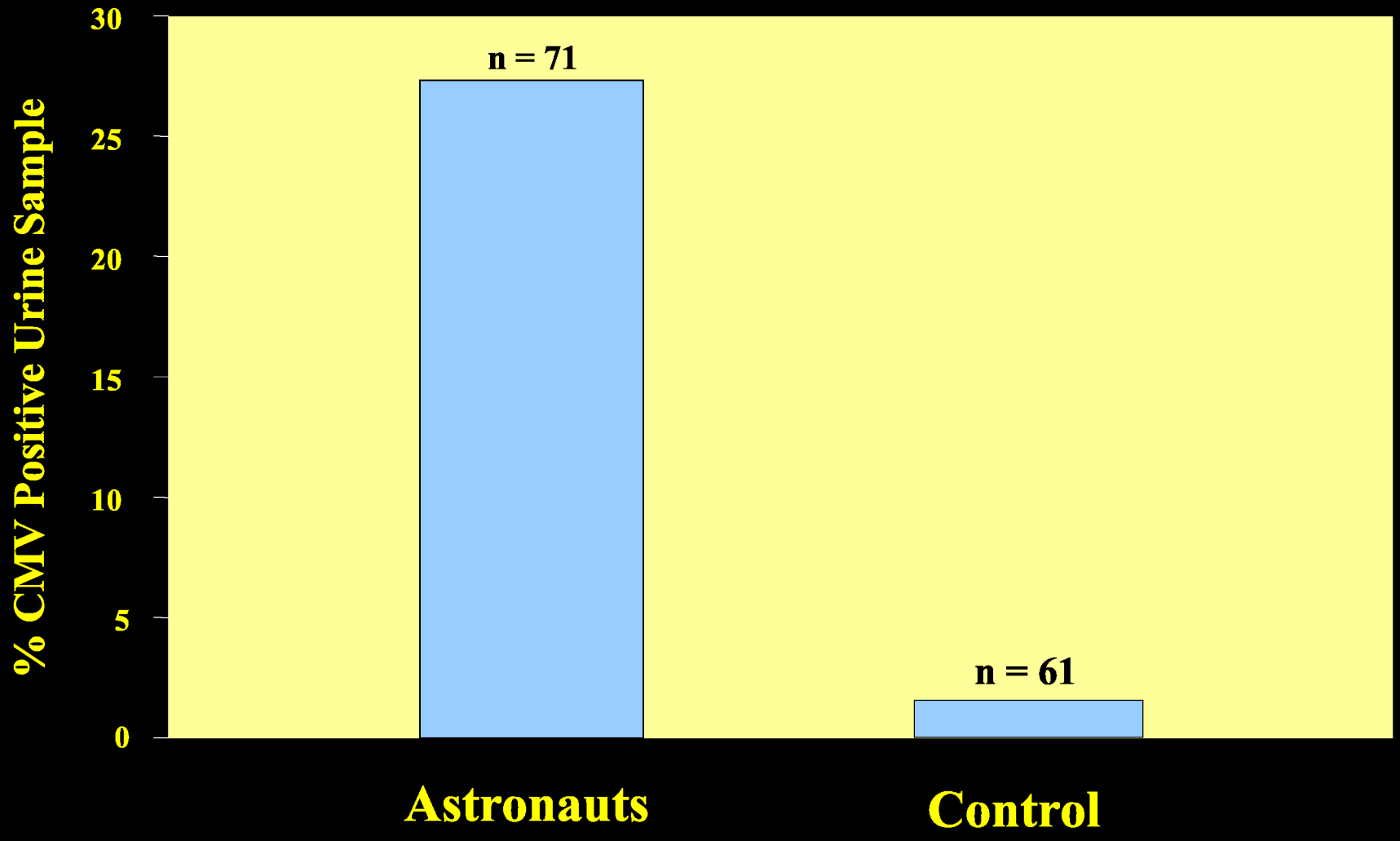
[a]Legend (+++ = highly expressed; ++ = moderately expressed; + = low expression); [b]Immediate early/early.

Summary of Nested RT-PCR Analysis of EBV Gene Expression in Astronauts

				Latency I-III					IE/E[c] Replicative				Late Replicative	
Mission	Subject	Time[b]	EBER1	Qp	LMP2A	Cp/Wp	LMP1	EBNA2	BZLF1	BHRF1	SM	Fp	BALF5	gp220
Shuttle	1	L-10	+++	+			+			+				
		R+0	+++				+							
	2	L-10	+++							+				
		R+0	+++											
	3	L-10	+++						+					
		R+0	+++	+					+					
	4	L-10	+++				+							
		R+0	+						+	+++				
	5	L-10	+							++				
		R+0	+++							++				
	6	L-10	+++						+	+++				
		R+0	+++	++						+++				
ISS	1	L-10	+++	+			+			++				
		R+0	+++	+	+					+++	+++		++	+++
	2	L-10	+++											
		R+0	+++	+			+	+++			+++			
	3	L-10	+++								++			
		R+0	+++			+++				+++				
	4	L-10	+							+++	+++			
		R+0	+++					+++		+++	+++	+		+++
	5	L-10	+++	+						+++				
		R+0	+++	+			+++	+++		+++		+	+++	+++
	6	L-10	+++		+	+++		+++		+++				
		R+0	+++	+					+	+++			+++	+++

[a]Legend (+++ = highly expressed; ++ = moderately expressed; + = low expression). [b]Collection time: Launch minus 10-days (L-10); Recovery/landing day (R+0). Average Shuttle flight = 11 days; average ISS mission = 180 days; [c]Immediate early/early.

Space Shuttle: CMV Frequency



Incidence of Shingles and Post Herpetic Neuralgia (PHN)

Shingles: Reactivation of VZV producing blisters in dermatomal region
- **Pain can be excruciating**

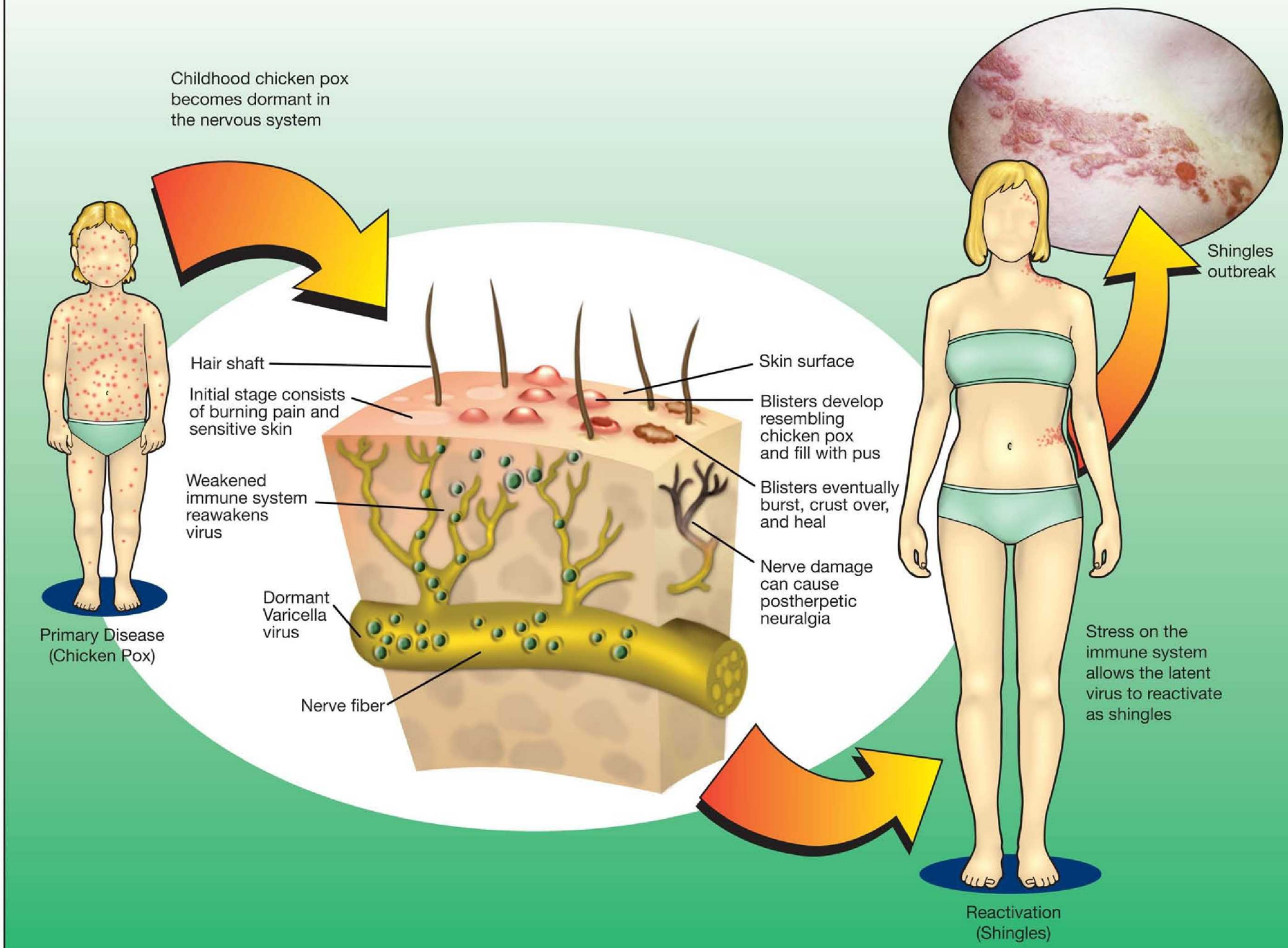
PHN: Prolonged, sometimes incapacitating, lasting weeks, months, or years.

"In extreme cases, PHN can be worse than death."

CDC

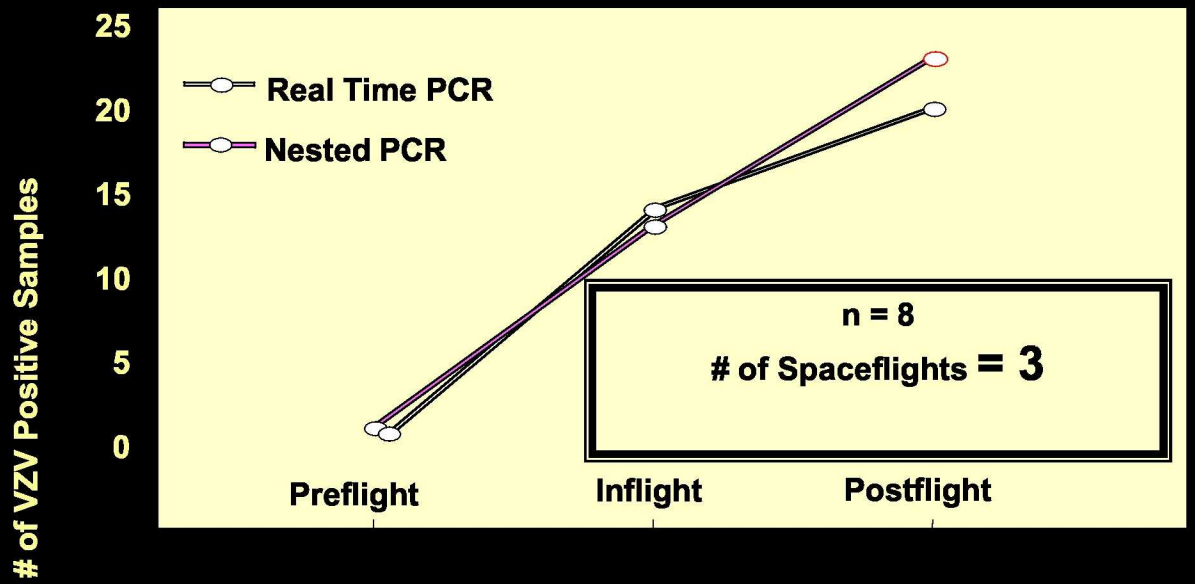
- **One million cases of shingles per year**
- **Risk of shingles increases >10-fold with age**
- **Lifetime risk of developing zoster: 25-30%**
- **100,000 to 200,000 cases of PHN per year**

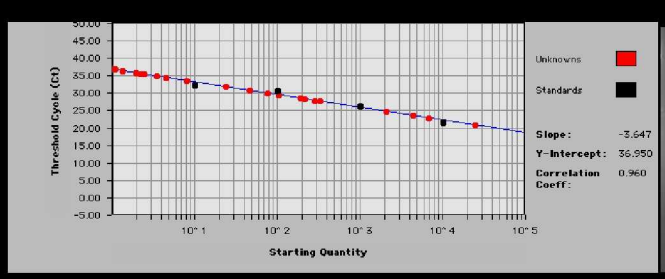


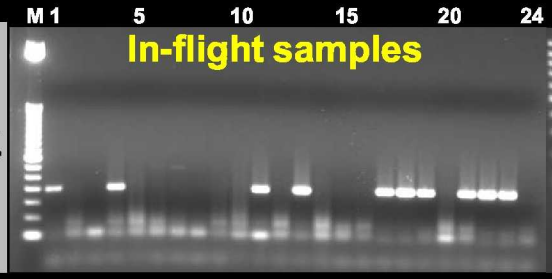


Childhood chicken pox becomes dormant in the nervous system
Primary Disease (Chicken Pox)
Hair shaft
Initial stage consists of burning pain and sensitive skin
Weakened immune system reawakens virus
Dormant Varicella virus
Nerve fiber
Skin surface
Blisters develop resembling chicken pox and fill with pus
Blisters eventually burst, crust over, and heal
Nerve damage can cause postherpetic neuralgia
Shingles outbreak
Stress on the immune system allows the latent virus to reactivate as shingles
Reactivation (Shingles)

First Report Of VZV DNA In Astronauts'Saliva

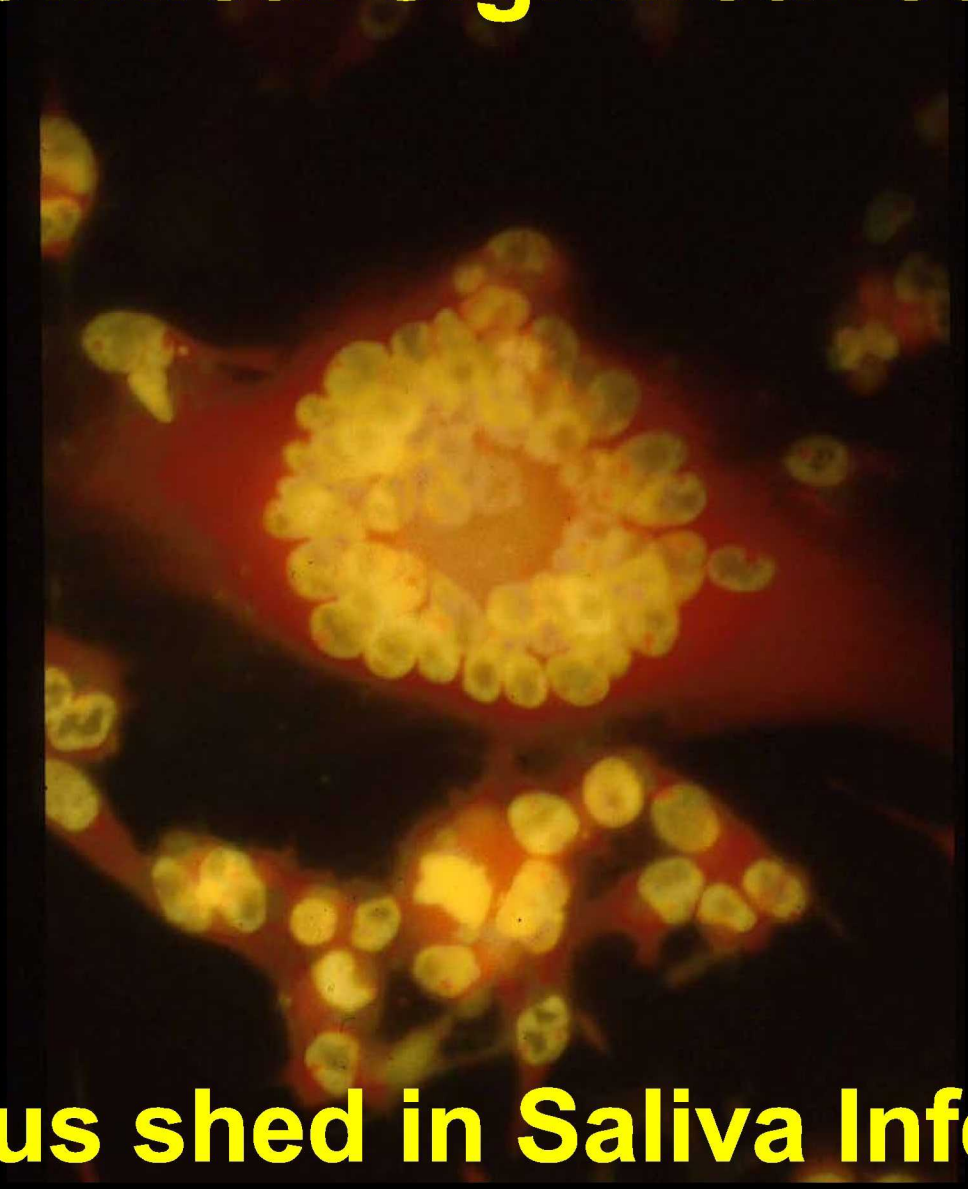
Mehta et al., J Medical Virology, 2004





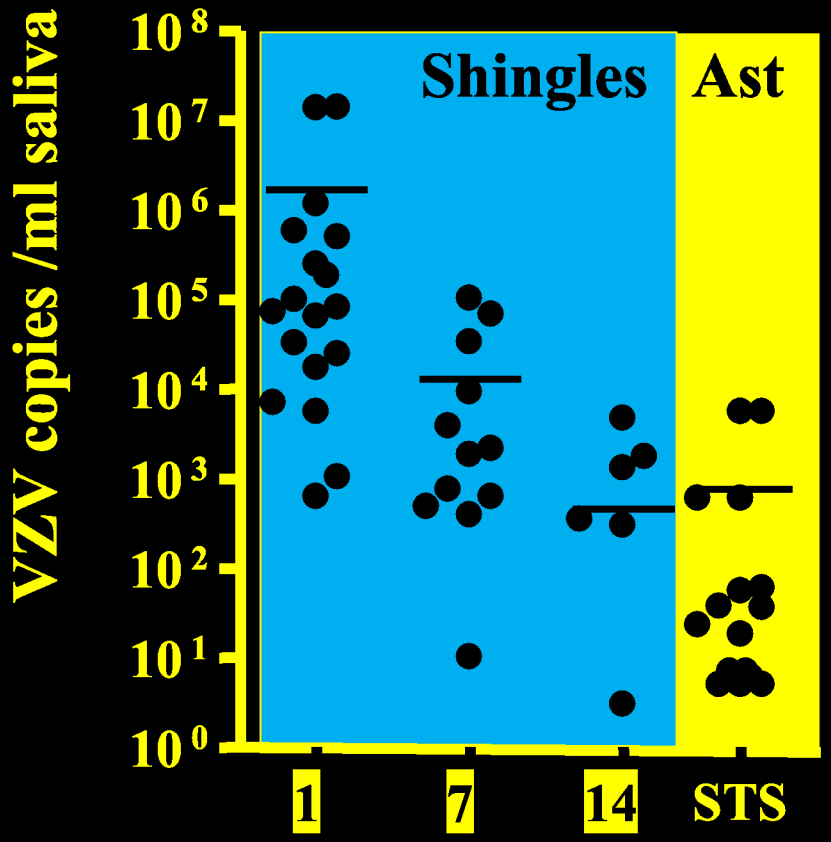


Clinical Significance?

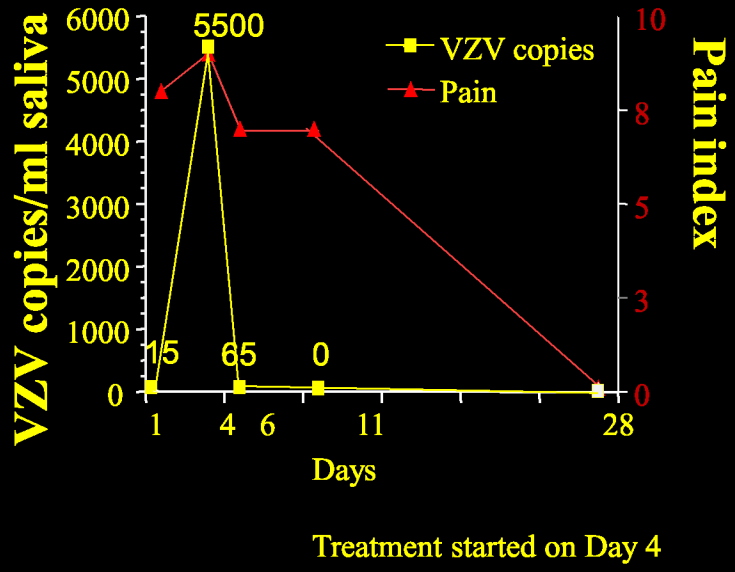


Is the Virus shed in Saliva Infectious?

Salivary VZV In Shingles Patients & Astronauts

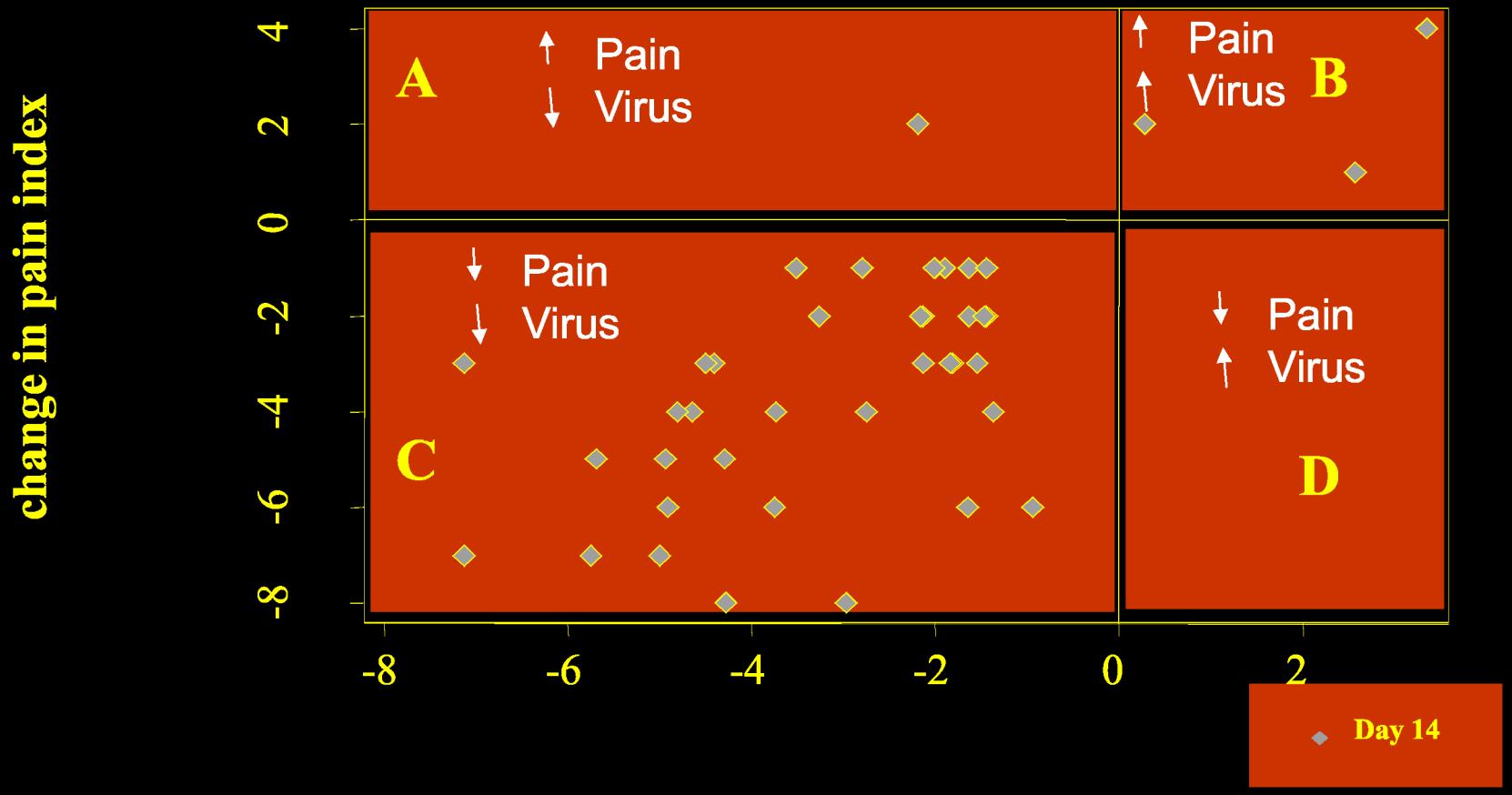


VZV copies in saliva of a 21 yr old patients with symptoms of Shingles.



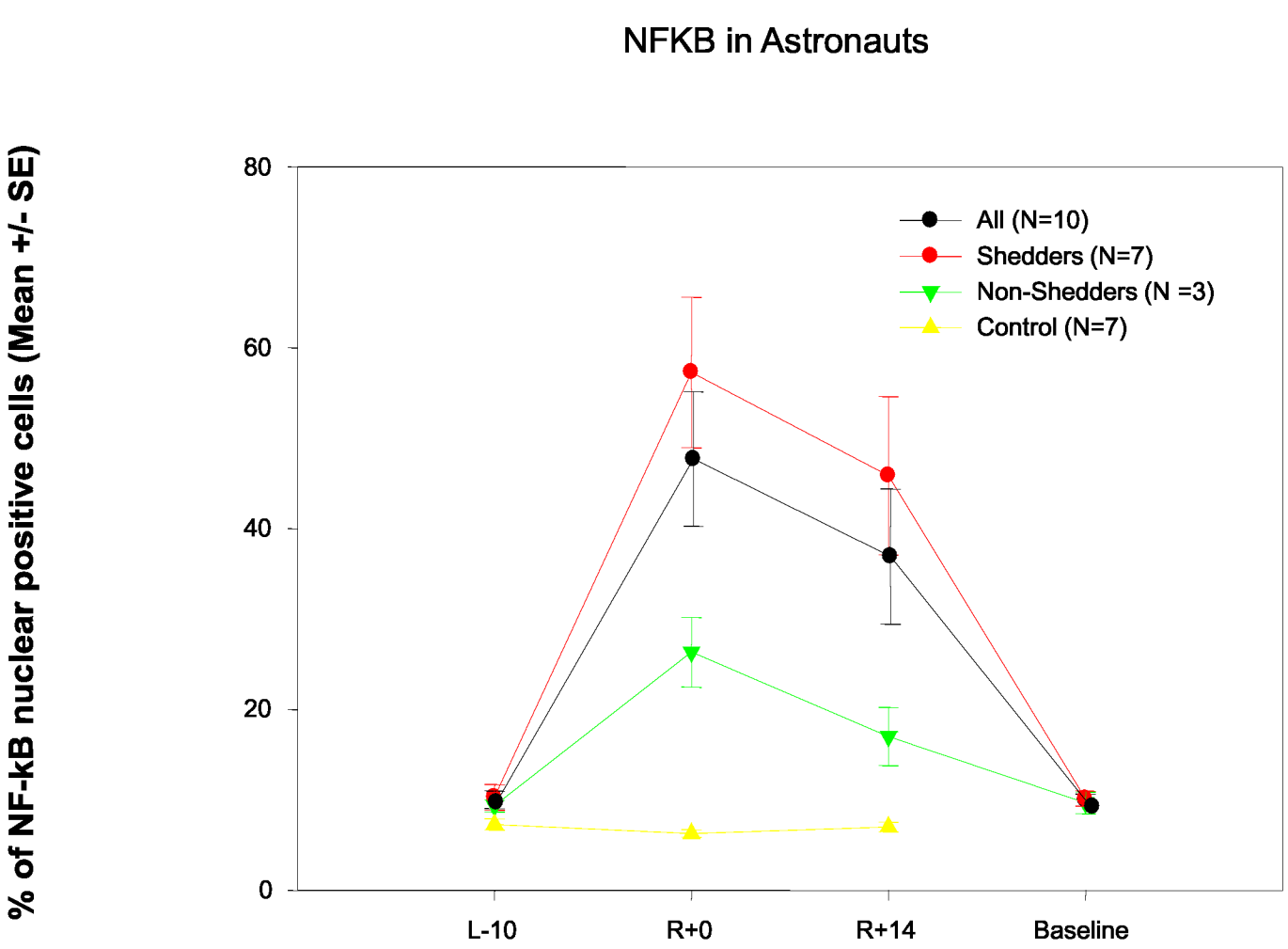
Mehta et al., 2008; Journal of Infectious Diseases

Change in Pain Index vs. Change VZV Copies After One Week of Treatment in Shingles Patients.

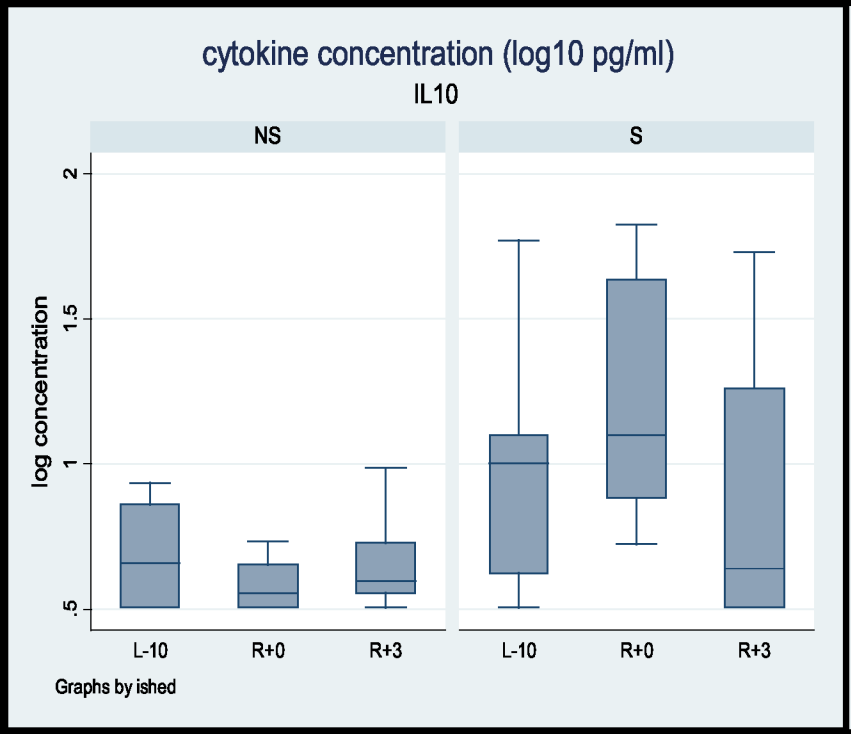


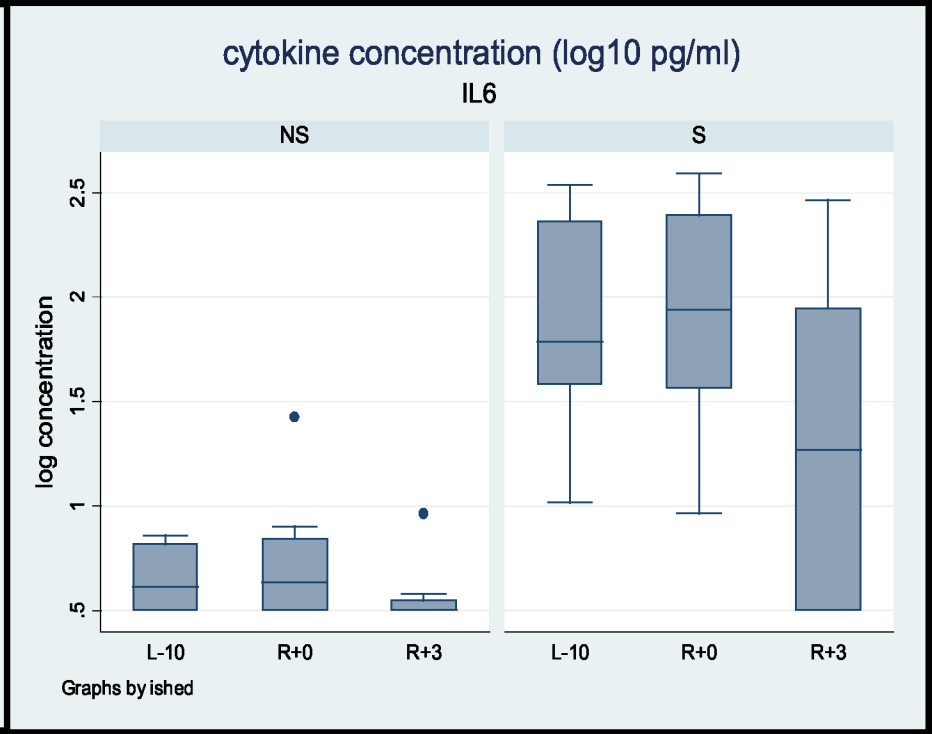
$\log_{10}$ Change in VZV Copies in Shingles Patients

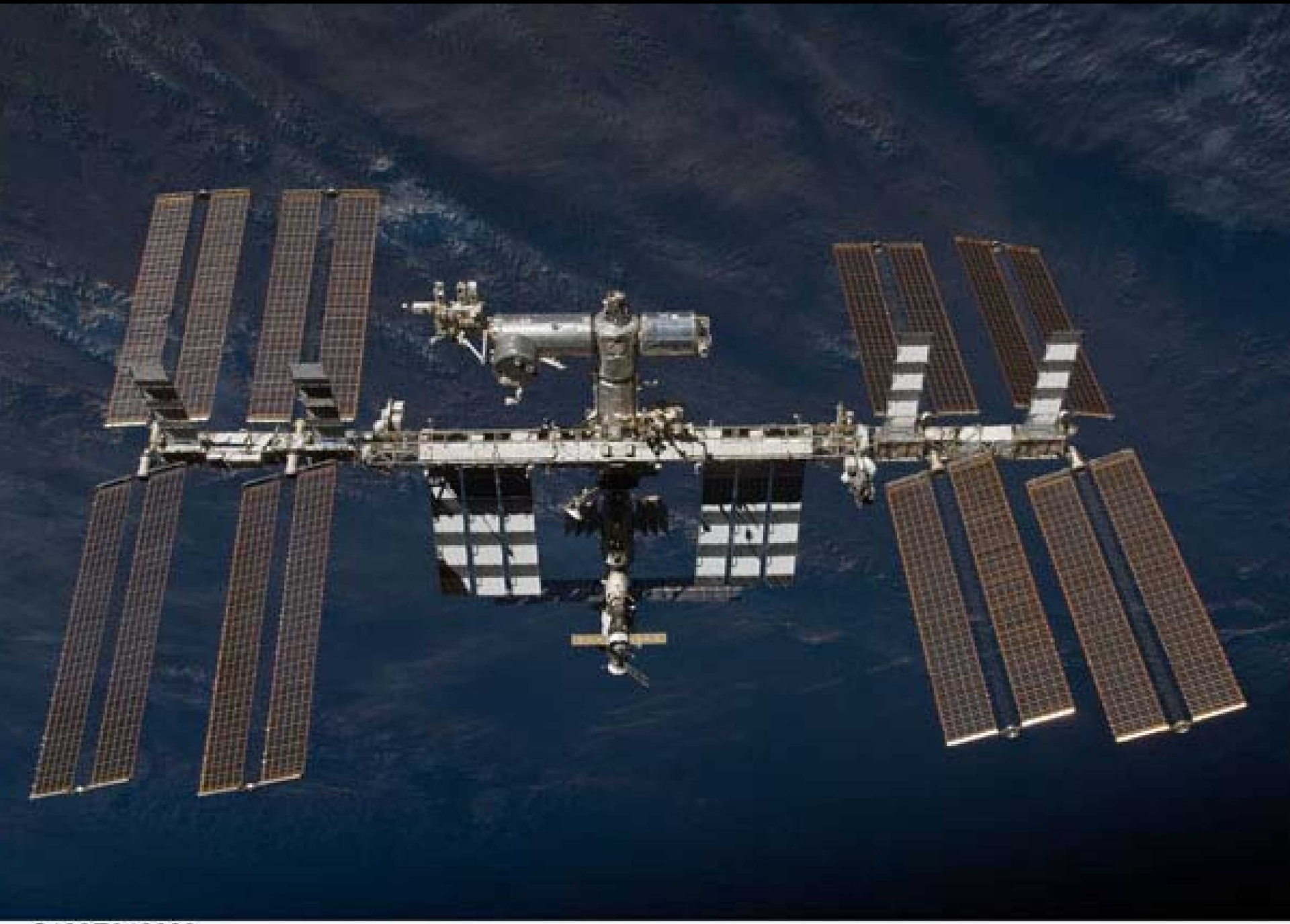
Note in almost every case, data points fell in quadrant C (both decreased)


NFKB in Astronauts
% of NF-kB nuclear positive cells (Mean +/- SE)
80
60
40
20
0
All (N=10)
Shedders (N=7)
Non-Shedders (N =3)
Control (N=7)
L-10
R+0
R+14
Baseline

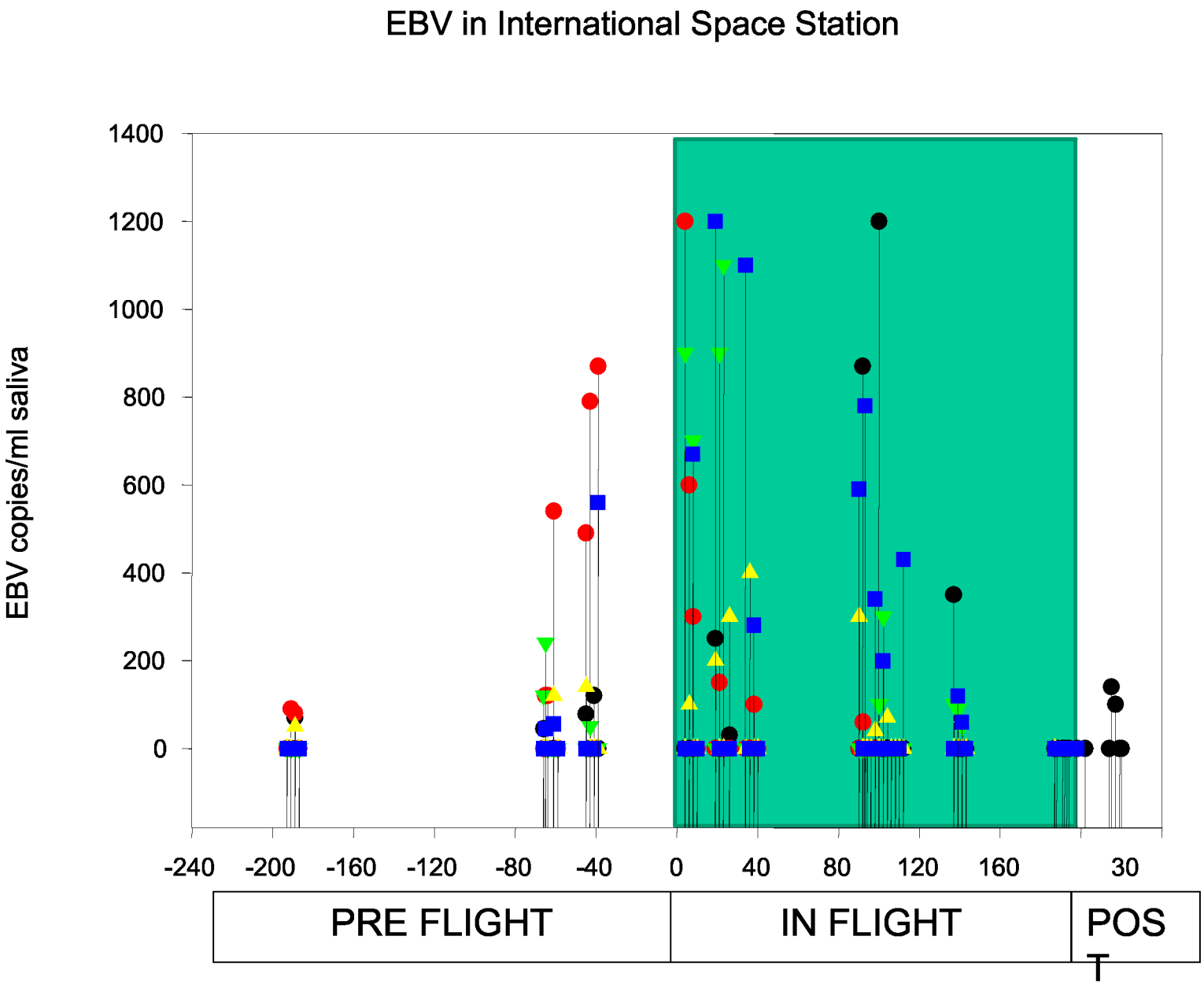
Cytokines

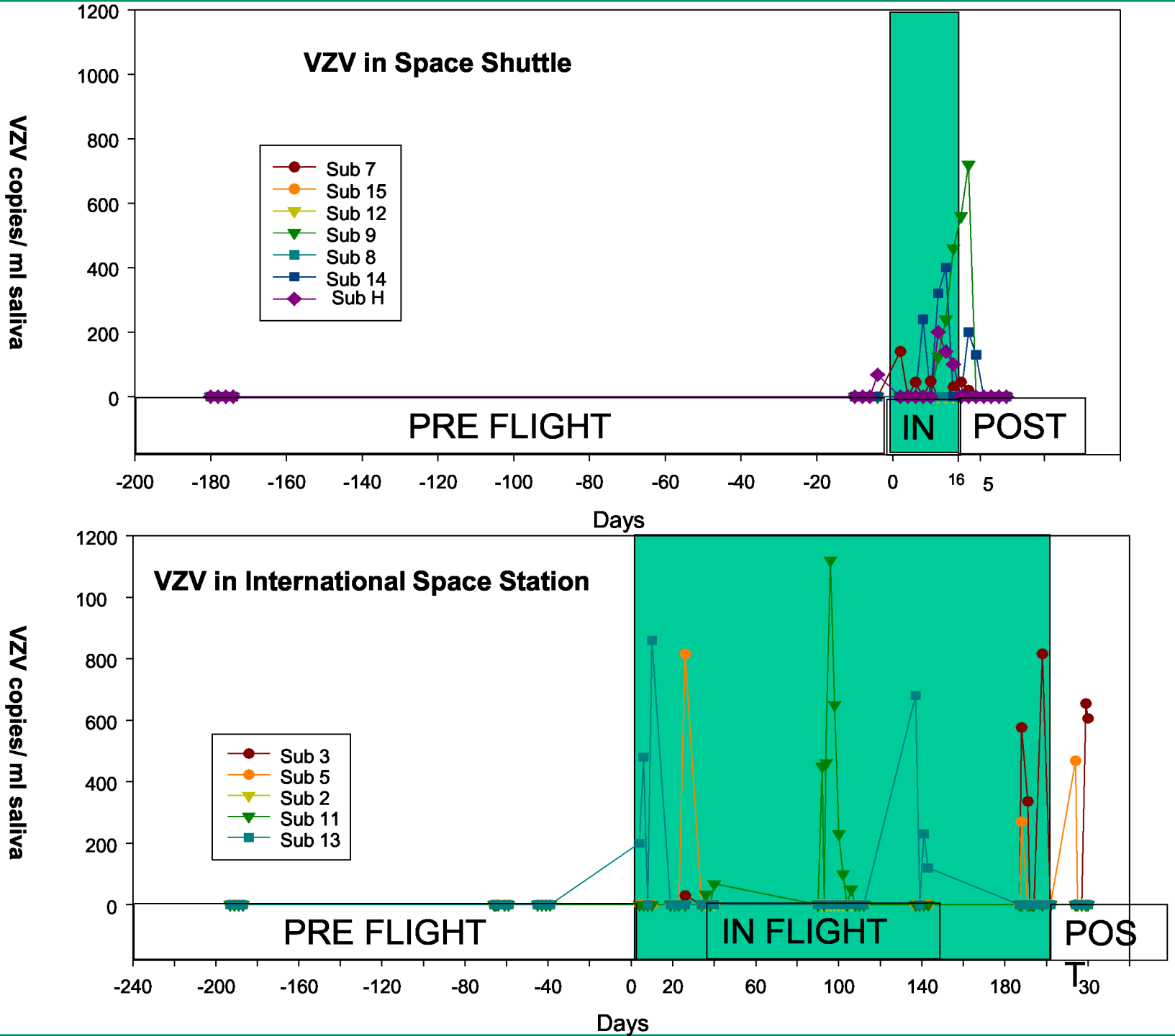

cytokine concentration (log10 pg/ml)
IL10
NS
S
log concentration
.5
1
1.5
2
L-10
R+0
R+3
L-10
R+0
R+3
Graphs by ished


cytokine concentration (log10 pg/ml)
IL6
NS
S
log concentration
.5
1
1.5
2
2.5
L-10
R+0
R+3
L-10
R+0
R+3
Graphs by ished



S130E012020


EBV in International Space Station
EBV copies/ml saliva
1400
1200
1000
800
600
400
200
0
-240
-200
-160
-120
-80
-40
0
40
80
120
160
30
PRE FLIGHT
IN FLIGHT
POST


VZV in Space Shuttle
VZV copies/ ml saliva
1200
1000
800
600
400
200
0
Sub 7
Sub 15
Sub 12
Sub 9
Sub 8
Sub 14
Sub H
PRE FLIGHT
IN
POST
-200
-180
-160
-140
-120
-100
-80
-60
-40
-20
0
16
5
Days
VZV in International Space Station
VZV copies/ ml saliva
1200
100
800
600
400
200
0
Sub 3
Sub 5
Sub 2
Sub 11
Sub 13
PRE FLIGHT
IN FLIGHT
POS
-240
-200
-160
-120
-80
-40
0
20
60
100
140
180
T30
Days

CONCLUSIONS

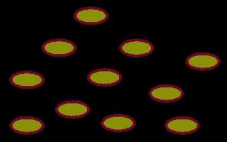
- Space flight is a unique stress model.
- Antarctic Science Stations model many aspects of space flight.
- Stress associated with space flight results in increased reactivation of EBV, CMV, and VZV.
- Viral reactivation in astronauts appears to be linked to duration in space (stress/microgravity?).
- Space flight-associated stress manifested through the HPA-axis result in increased stress hormones, reduced CMI, and increased viral reactivation.
- Viral reactivation may be used as an early predictor of impending medically significant changes in the immune response.

VZV can reactivate subclinically in healthy individuals after acute stress.

Changes in Microbial Pathogen Characteristics

Collaborative Studies

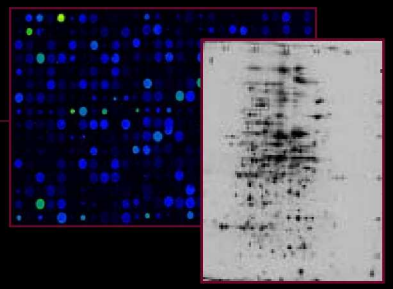
PI: Dr. Cheryl Nickerson, Arizona State University



Salmonella typhimurium

Rotating Wall Vessel bioreactor reproduces aspects of microgravity (Low fluid shear, low mass diffusion)

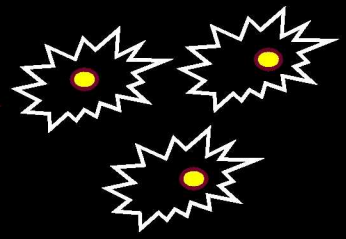
Salmonella **grown in spaceflight analogues displayed increased virulence**



Salmonella **grown in spaceflight analogues altered their gene and protein expression**

Classic virulence genes down-regulated

Ion response genes/pathways



Salmonella **grown in spaceflight analogues altered their response to environmental stresses**

Macrophage, acid, thermal, osmotic, oxidative

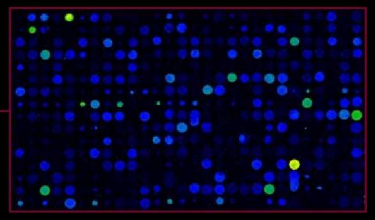
Nickerson et. al., 2000, Infect. Immun. 68:3147-3152; Wilson, et al., 2002, Proc. Natl. Acad. Sci. USA. 99:13807-13812; Wilson, et al., 2002, Appl. Environ. Microbiol. 68:5408-5416; Nickerson, et al., 2004, Microbiol Mol Biol Rev, 68:345-361.

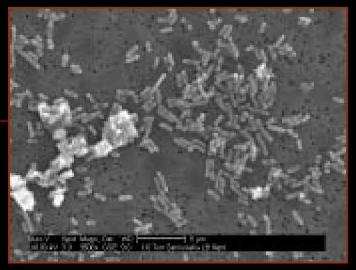
MICROBE

Shuttle Atlantis, STS-115, launch Sept 9, 2006

Salmonella enterica Typhimurium experimental design and results

In-flight hardware





Salmonella grown during spaceflight displayed increased virulence in rich media

- Killed mice faster and killed mice at lower doses than identical bacterial cultures grown on the ground
- Virulence change dependent on the growth media

Salmonella grown during spaceflight altered their gene expression

- 167 genes differentially regulated
- Ion response genes/pathways
- Identification of the global molecular regulator, *hfq*, ("master switch") of spaceflight induced cellular responses

Salmonella grown during spaceflight showed the presence of a material resembling a biofilm

- Biofilms are important in disease causing potential and vehicle system failure

* ***Synchronous ground controls maintained under identical conditions as those on-board Shuttle*** *- ground and in-flight hardware loaded with same sample.*

Wilson et al., 2007, Proc. Natl. Acad. Sci. USA. 104(41):16299-304; Wilson et al., 2008, PLOS One 3(12): e3923

MDRV

Shuttle Endeavour, STS-123, launch March 11, 2008
Experimental design and results

In-flight hardware

Confirmed the effect of spaceflight on *Salmonella* virulence observed in MICROBE

Demonstrated a "spaceflight response" regardless of culture media

Established a link between the spaceflight response and media composition

Ion levels can be modulated to control spaceflight-associated virulence response of *Salmonella*

Phosphate ion sufficient to alter related pathogenesis responses in spaceflight analogue model.

In combination with MICROBE results, MDRV is showing a common conserved response in many microorganisms

MICROBE and MDRV also evaluating organisms, such as *Pseudomonas aeruginosa* and *Candida albicans*

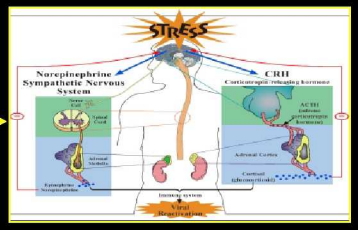
* ***Synchronous ground controls maintained under identical conditions as those on-board Shuttle*** - *ground and in-flight hardware loaded with same sample.*

Wilson et al., 2008, PLOS One 3(12): e3923

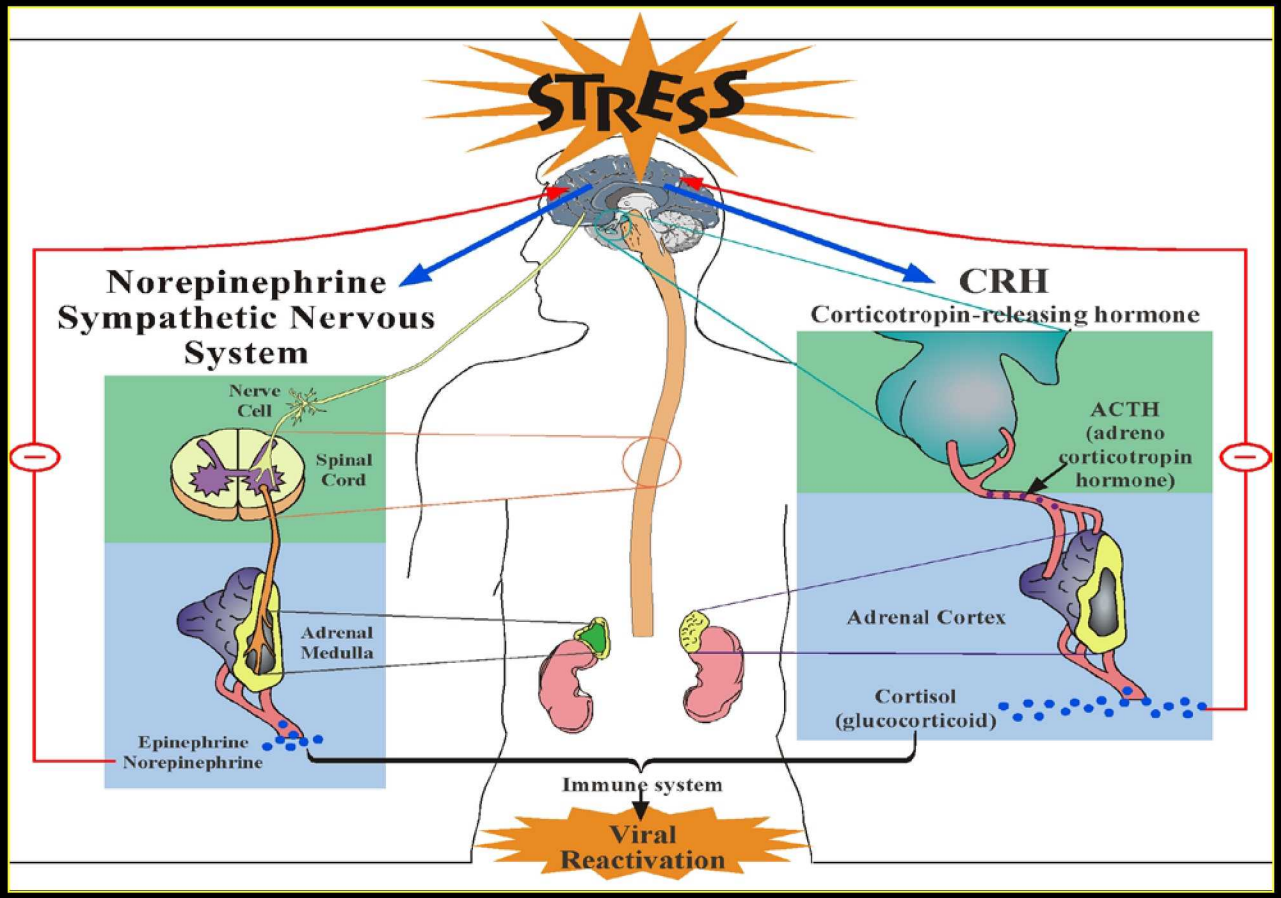
Overview

Increased Stress

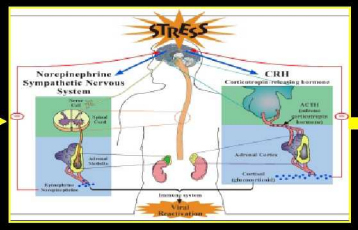
Increased Stress



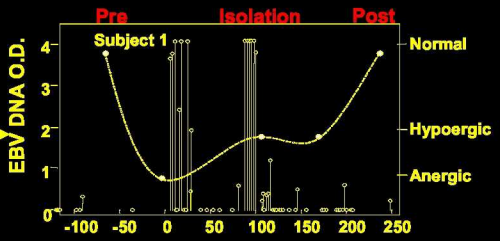
Increased Stress Hormones



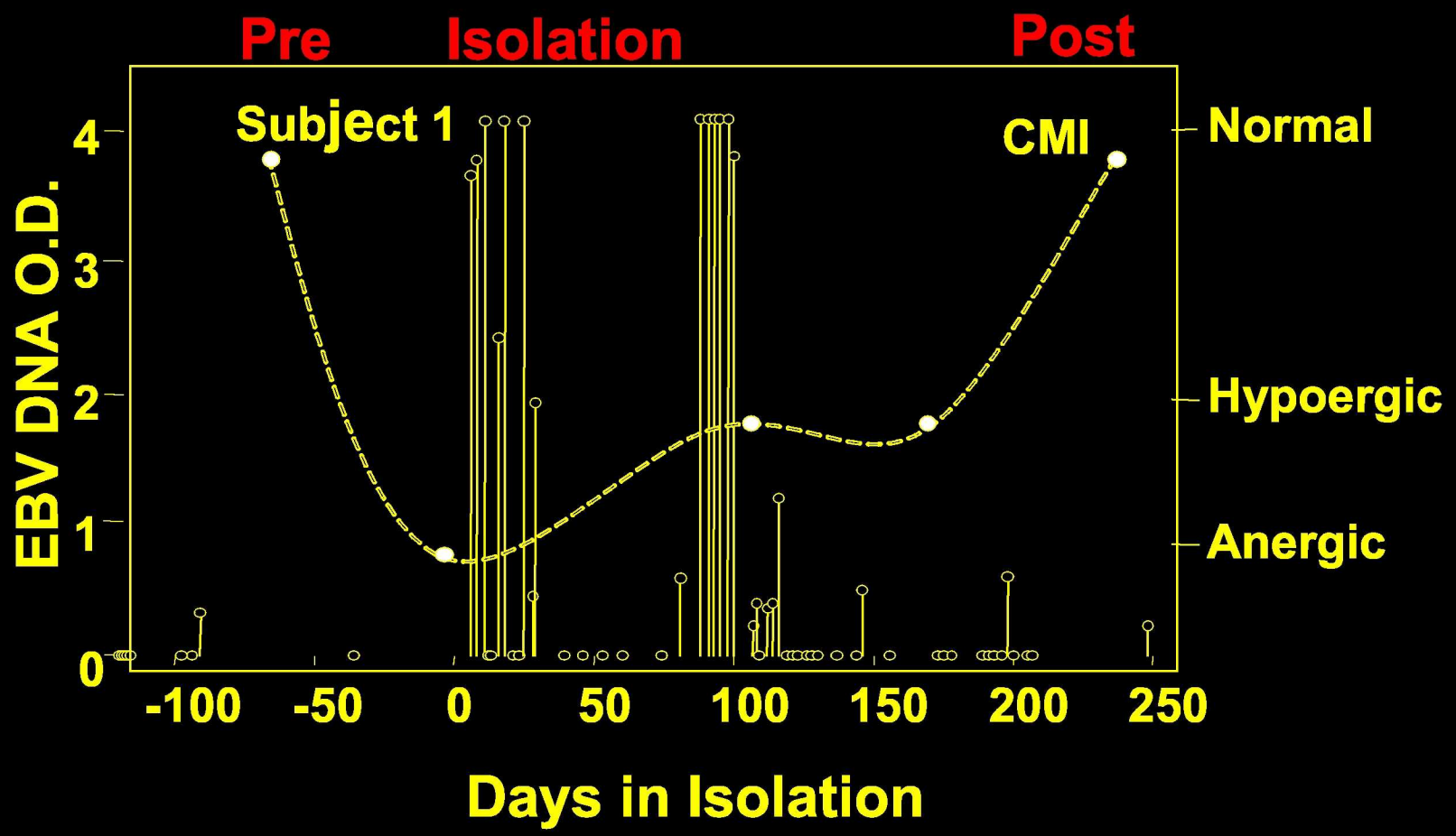
Increased Stress



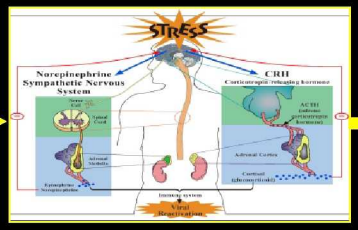
Increased Stress Hormones



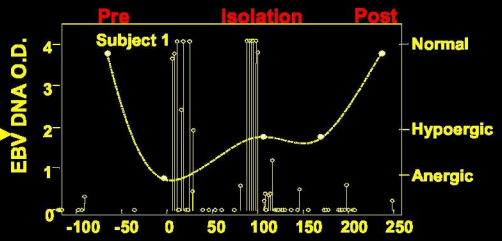
Decreased Immunity



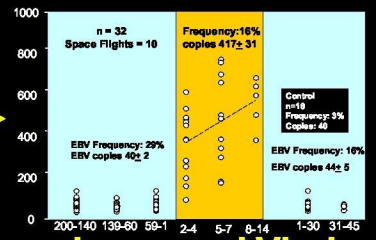
Increased Stress



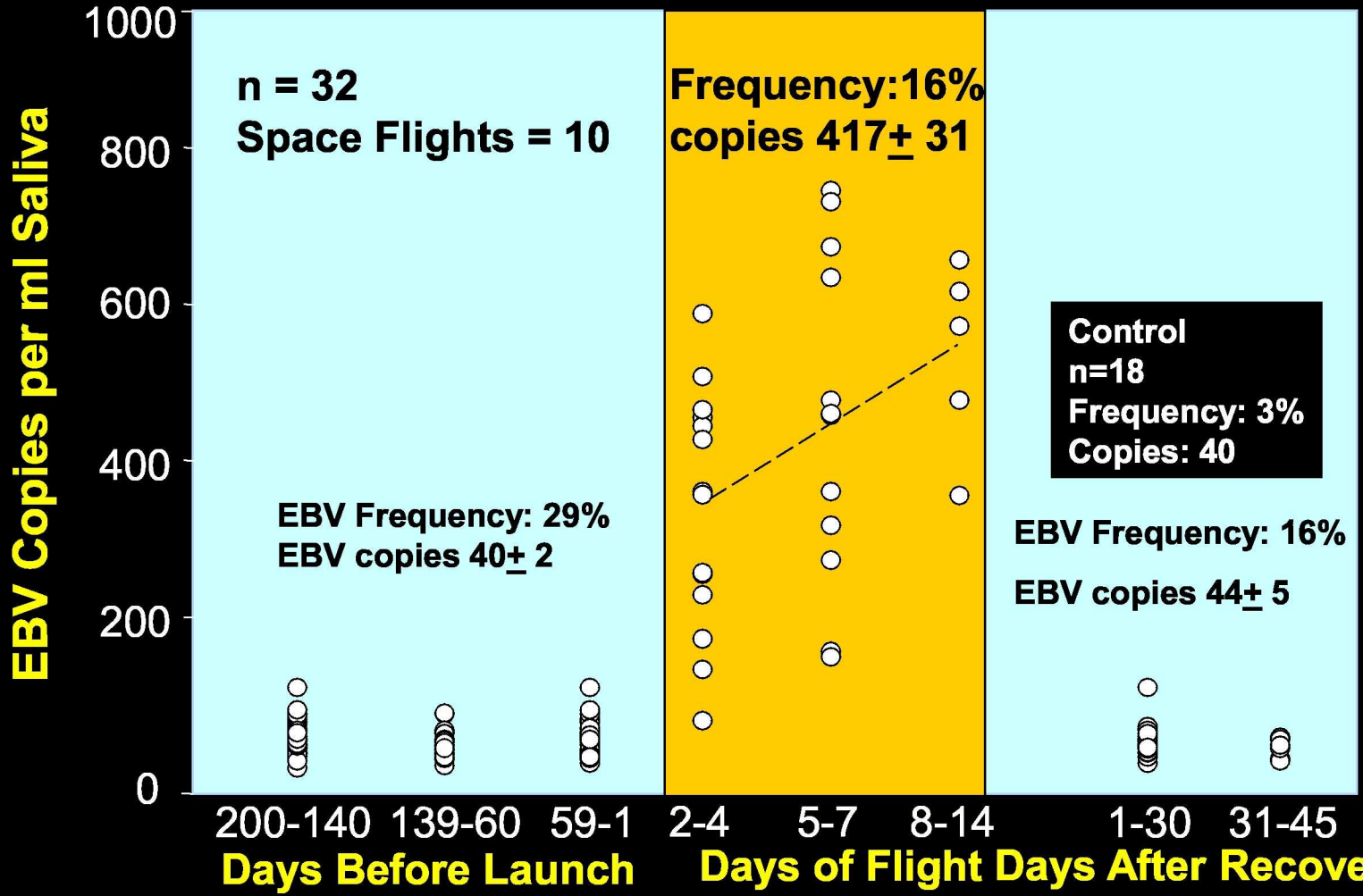
Increased Stress Hormones



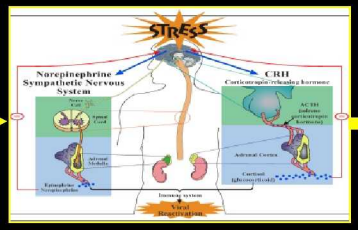
Decreased Immunity



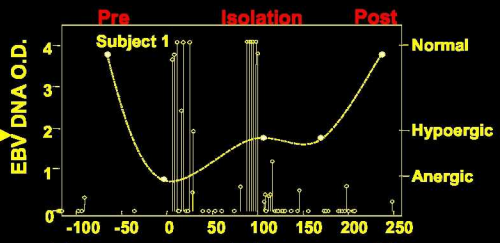
Increased Viral Reactivation



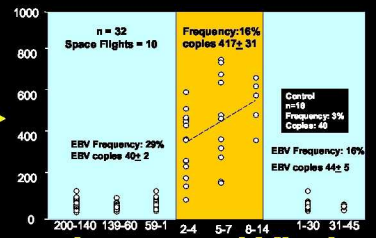
Increased Stress



Increased Stress Hormones



Decreased Immunity

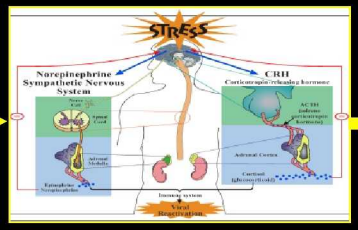


Increased Viral Reactivation

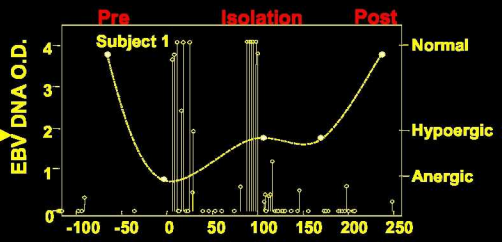
Disease Risks

- Shingles
- Ocular Herpes
- Hepatitis
- Tumors
- Mononucleosis
- Skin Lesions

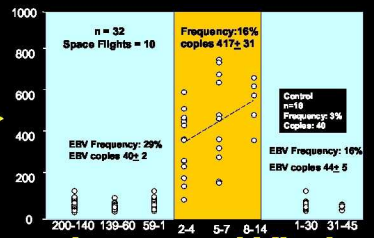
Increased Stress



Increased Stress Hormones



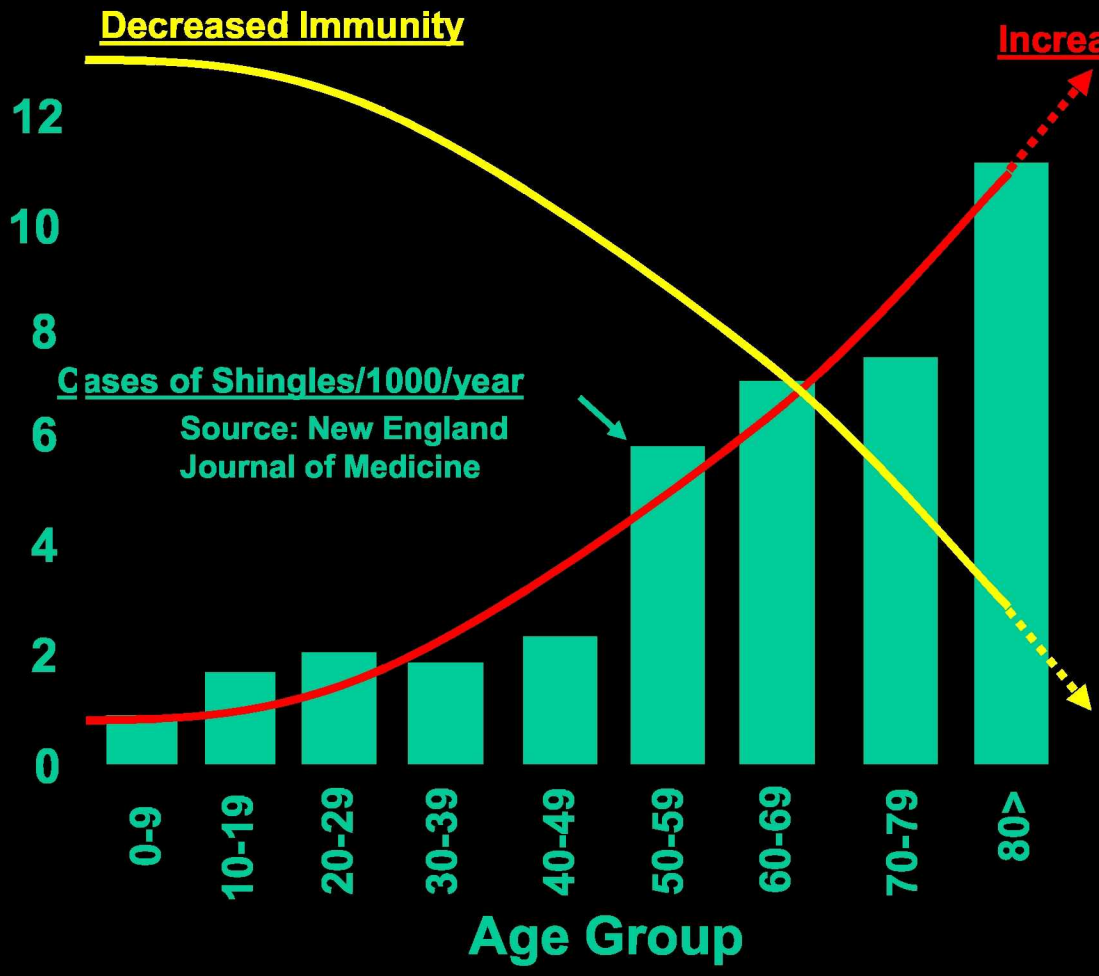
Decreased Immunity



Increased Viral Reactivation

Disease Risks

- Shingles
- Ocular Herpes
- Hepatitis
- Tumors
- Mononucleosis
- Skin Lesions



Collaborators

- Microbiology Laboratory, JSC-NASA, Houston, TX
- Stephen K. Tyring, M.D., Ph.D., UTHSC, Houston, TX
- Don Gilden, M.D., UCHS, Denver, CO
- Randall J. Cohrs, Ph.D., UCHS, Denver, CO
- Ronald Glaser, Ph.D., Ohio State University, Columbus, OH
- Mark Laudenslager, Ph.D., UCHS, Denver, CO
- Desmond J. Lugg, M.D., Australian Antarctic Division, Hobart, Australia
- Raymond P. Stowe, Ph.D., UTMB, Galveston, TX
- Janet S. Butel, Ph.D., NSBRI/Baylor College of Medicine, Houston, TX
- Indresh Kaur, Ph.D., M.D. Anderson, Houston, TX
- Alan Feiveson, JSC-NASA, Houston, TX

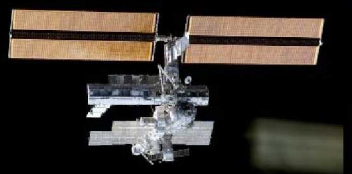

Questions?

IMPACT OF STRESS

Low **High**

STRESS LEVELS AND MISSION IMPACT

Space Shuttle



ISS

Mir

Moon

Mars

NFkB

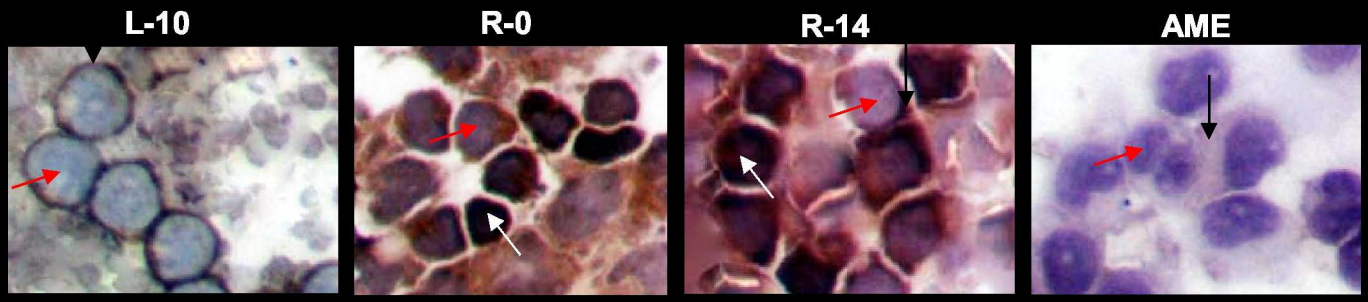
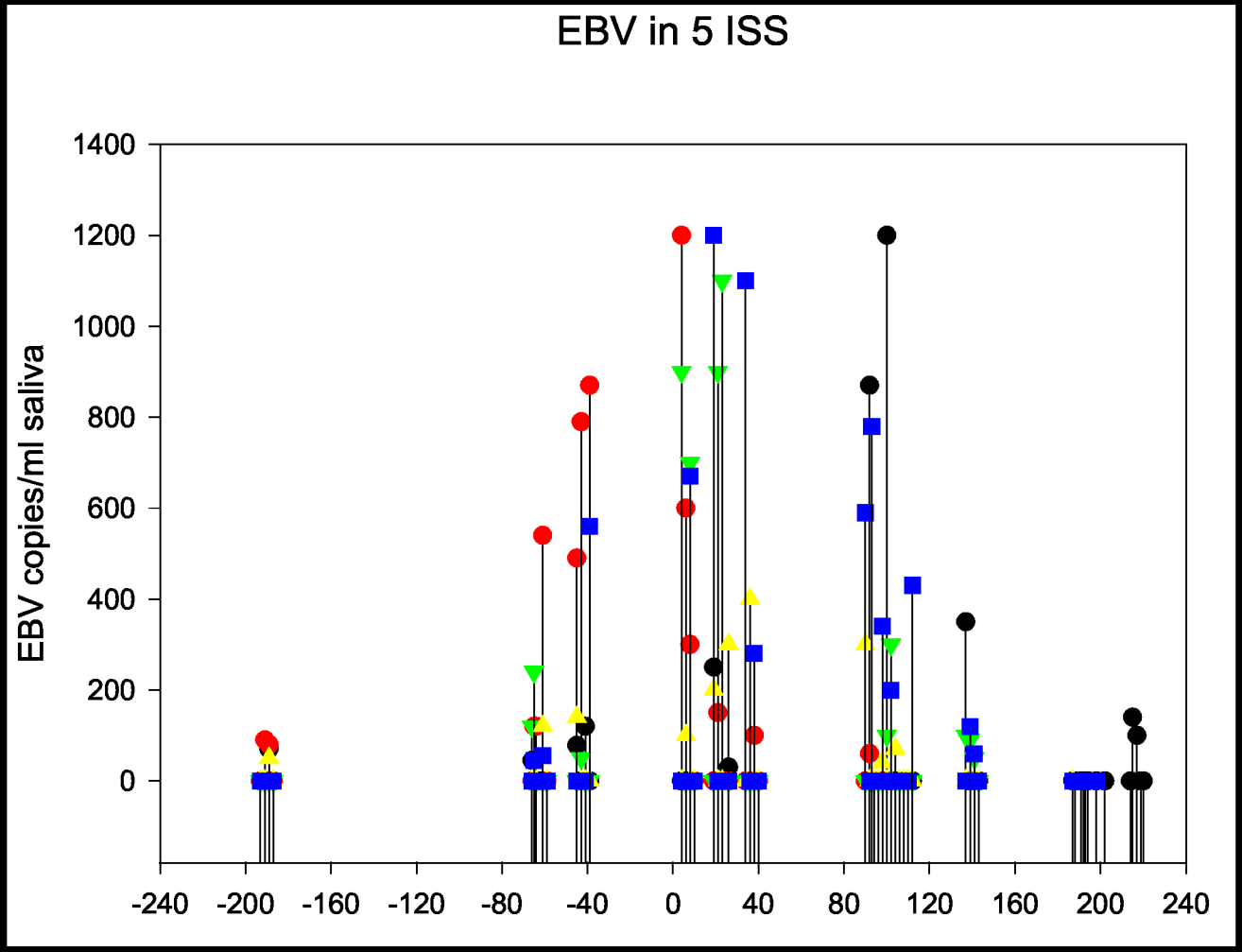


Figure 1: NF-κB activation in the PBMC of astronauts at different time points. Cells from astronauts at different time points were collected were analyzed for nuclear p65 as described in Materials and Methods.
In the cytoplasm (that is; in normal conditions), NF-κB consists of a heterotrimer of p50, p65, and IκBα. When it gets activated, that is; under stressed or diseased conditions IκBα undergo phosphorylation and separated from the p65-p50 complex. Then the p65-p50 subunit translocated to the nucleus, attach to specific regions of DNA (that is; the promoters of some genes) and initiates gene transcription that are involved in inflammation and cancer. In this figure black arrow represents inactivated form of NF-κB (in the cytoplasm), white arrow represents nuclear translocation of NF-κB (p65-p50 complex) and red arrow represents hematoxylene staining in the nucleus of the cells that have inactivated form of NF-κB.


EBV in 5 ISS
EBV copies/ml saliva
1400
1200
1000
800
600
400
200
0
-240
-200
-160
-120
-80
-40
0
40
80
120
160
200
240

CMV In Space Shuttle And International Space Station Crewmembers

	# of Space Shuttle crewmembers shed CMV	# of International Space Station crewmembers shed CMV
Before flight		
180 d before Launch	0/7	0/5
45 d before Launch	0/7	0/5
10 d before Launch	3/7	not done
After flight		
At Landing	4/7	4/5
14 d after landing	4/7	not done
30 d after Landing	not done	4/5
Overall	4/7	4/5

Conclusions

1. Four of the eight herpes viruses reactivate in response to short term shuttle and long term ISS flights.

2. Reactivation and shedding of EBV, CMV, and VZV on ISS was more pronounced and shed for longer time post flight than short duration shuttle flights.

3. Effects of stressors associated with spaceflight are mediated through the HPA axis and the SAM axis resulting in diminished cellular immunity.

4. Changes on circadian rhythms of cortisol and DHEA occur both ISS and SS crewmembers.

5. Spaceflight developed PCR technology has been transferred to Physicians' laboratories for diagnosis of Shingles and post herpetic neuralgia.

Summary of Nested RT-PCR Analysis of EBV Gene Expression[a] in Aging

Subject	EBER-1	Qp	Cp/Wp	LMP-1	EBNA-2	BZLF-1	SM	Fp	gp220
1	+++	+	+	+				+	+++
2	+++		+	+				+	++
3	+++	+	+	+				+	+++
4	+++	+		+		+		+	++
5	+++	+							+
6	+++	+	+	+				+	+++
7	+++			+				+	
8	+++	+							+++
9	+++			+				+	+
10	+++			+	+++		+++	+	+++
11	+++		+						

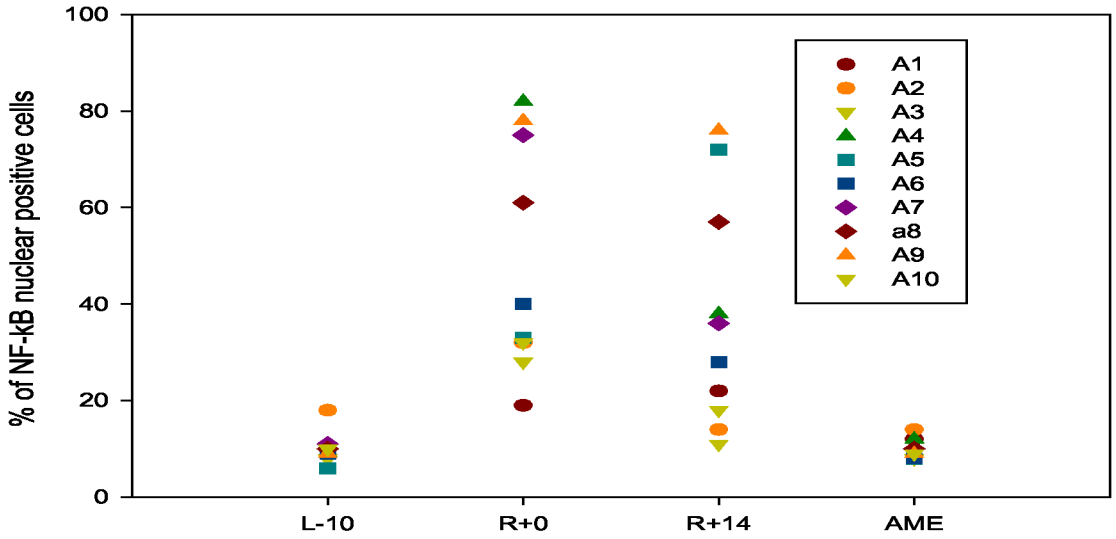
Note: accumulated data for multiple (2-3) timepoints for each elderly subject.
[a]Legend (+++ = highly expressed; ++ = moderately expressed; + = low expression)
[b]+ = EBV DNA present

CURRENT FOCUS: ON VZV

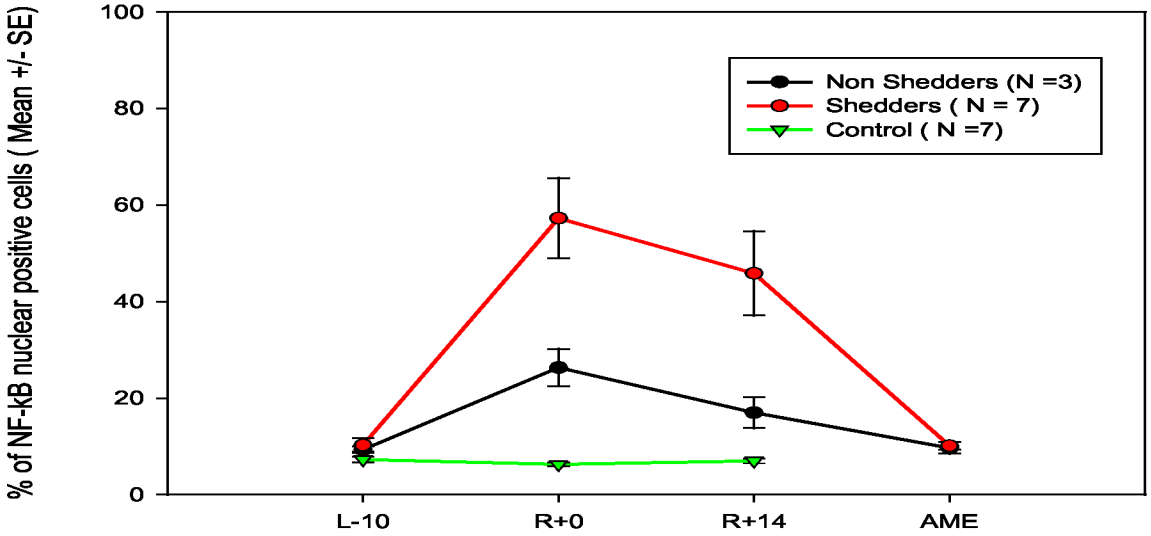
Unlike other neurotropic alphaherpesviruses in which primary infection is often asymptomatic, VZV (chickenpox) is characterized by malaise, fever, and an extensive vesicular rash.

The occurrence of VZV 2 days before space flight in a 47 year-old healthy astronaut from a pool of 81 physically fit astronauts prompted our search for subclinical VZV reactivation during times of stress.

NFKB in 10 astronauts before and after space flight



Mean +/- SE



PATHOGENS

Public Health

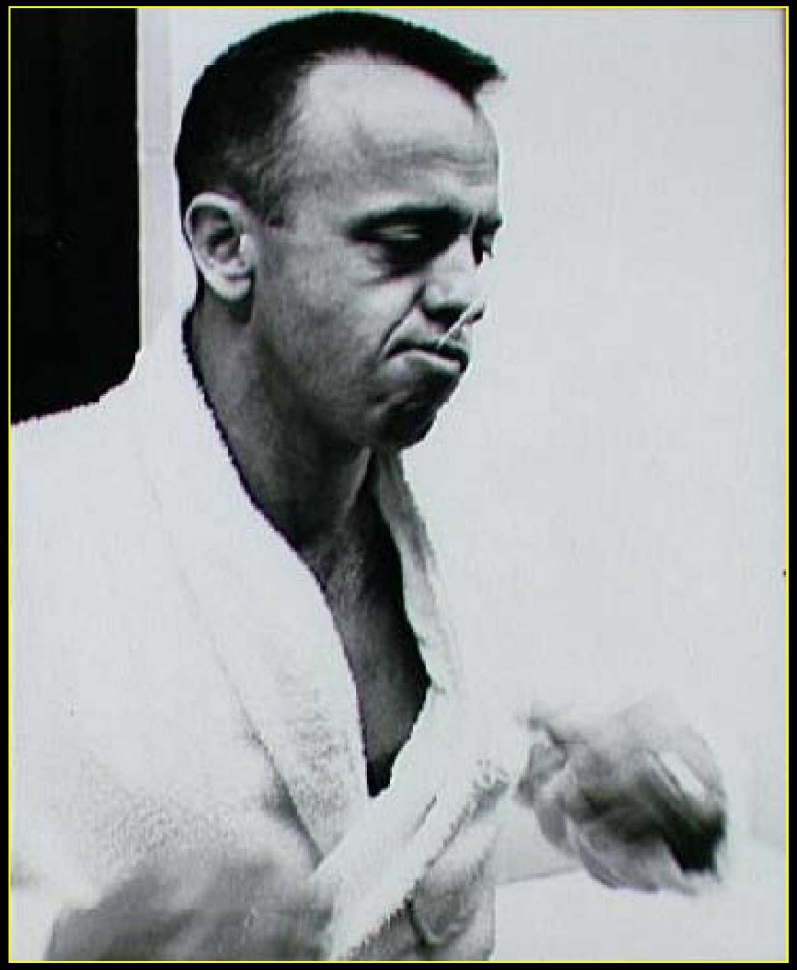
Mycobacterium tuberculosis
Helicobacter pyogenes
Staphylococcus aureus (MRSA)
Meningitis
STD's
Salmonella spp
Childhood diseases (e.g., measles)
Escherichia coli 0157: H7
HIV
HAV, HBV, HCV
Herpes viruses
Influenza (respiratory viruses)

Space Flight

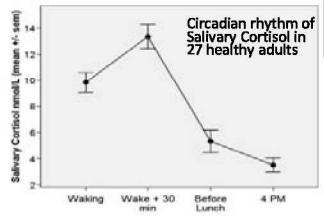
MRSA
Streptococci
Escherichia coli
Pseudomonas aeruginosa
Legionella pneumophila
Salmonella
Herpes viruses
Norovirus
Aspergillus
Penicillium
Candida
Giardia
Cryptosporidium

PREVENTIVE MEASURES

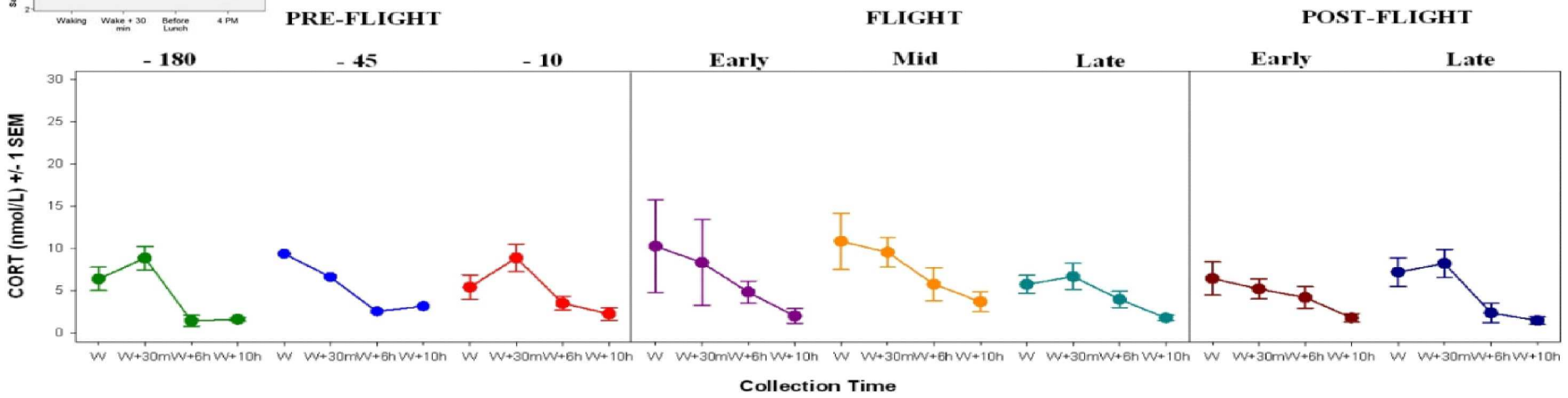
- Crew Physical Examinations
- Immunization
- Health Stabilization Program
- Quarantine
- Preflight Food Testing
- Payload Biosafety Evaluation
- Establishment of Acceptability Limits
- Systems Design
- Environmental Monitoring
- In-Flight Housekeeping
- In-Flight Diagnostic Capabilities
- Antimicrobials



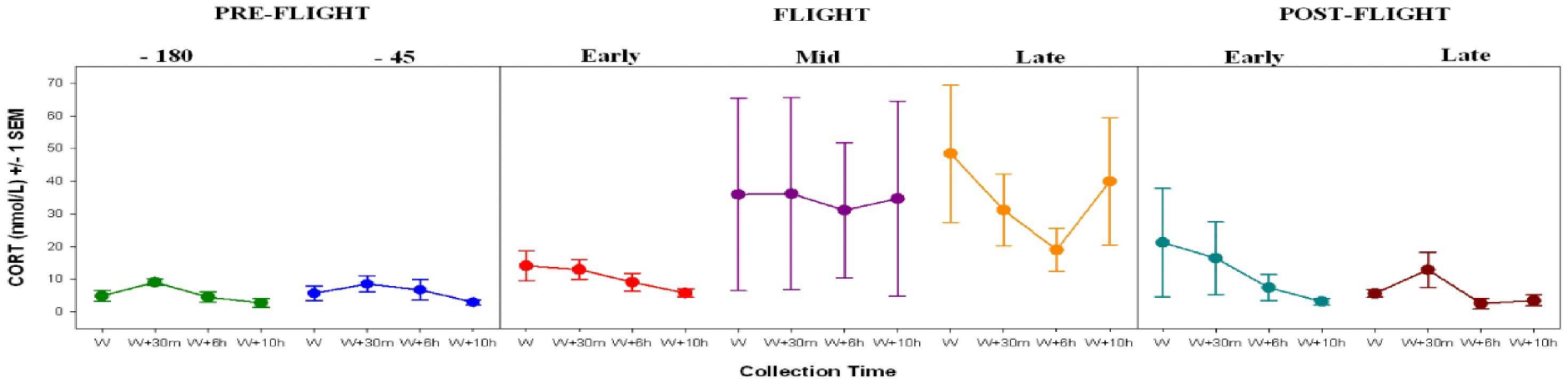
Salivary cortisol



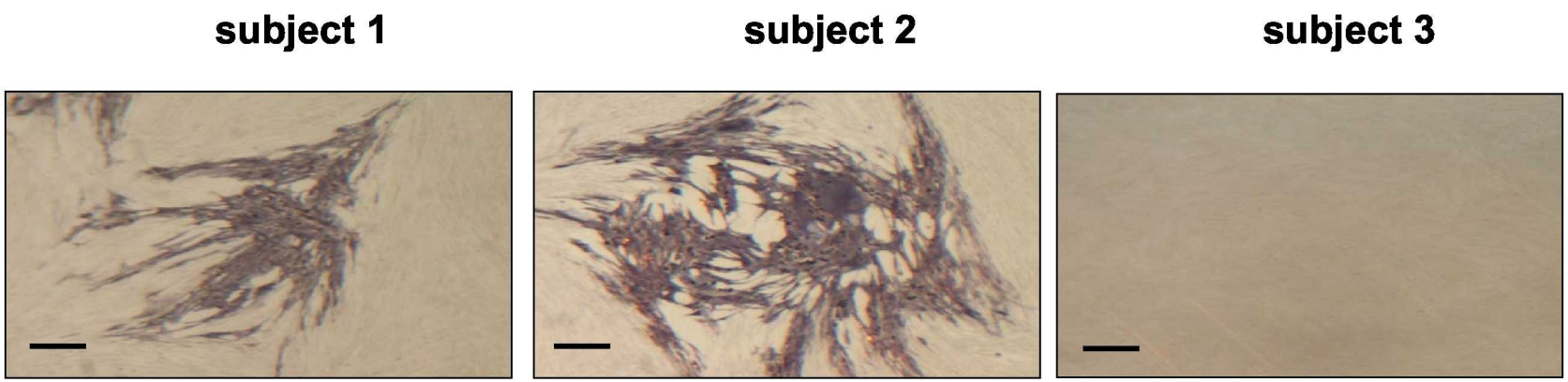
Space Shuttle



International Space Station



The decline in CMI to VZV associated with zoster led to the hypothesis that infectious VZV would also be present in the saliva of astronauts subjected to stress of spaceflight. Herein, not only was the detection of salivary VZV DNA associated with spaceflight validated, but also infectious virus was also detected in saliva. **This is the first demonstration of shed of infectious VZV in the absence of disease.**



Recovery of infectious VZV from astronaut saliva. Human lung fibroblast cells cultures were inoculated with saliva from astronauts obtained on day 2 after landing. Typical herpes virus plaques were seen in cultures inoculated with saliva from subjects 1 and 2, but not with saliva from subject 3. The plaques stained with anti-VZV antibody but not with anti-HSV-1 antibody (not shown). magnification bar = 0.2 mm.

Cohrs et al. J Medical Virology, 2008